TITLE

METHODS FOR TREATING AUTOIMMUNE AND CHRONIC INFLAMMATORY CONDITIONS USING ANTAGONISTS OF CD30 OR CD30L

CROSS REFERENCE TO RELATED APPLICATIONS

This application is a divisional of U.S. patent application 09/921,667 filed on August 3, 2001, now allowed, which claims the benefit of priority from U.S. patent application 60/224,079, filed August 8, 2000.

FIELD OF THE INVENTION

This invention relates generally to methods for treating autoimmune and inflammatory disorders that involve administering agents that block the interaction between CD30 and CD30L or IL-1 and IL-1R1, and also provides an animal model for testing the ability of a compound to treat inflammatory conditions that respond poorly to treatment with TNFα inhibitors.

BACKGROUND OF THE INVENTION

CD30 and its ligand, CD30L, are membrane proteins of the TNFR and TNF ligand superfamilies, respectively, and are expressed on various lymphoid and myeloid cells. CD30 was first described as an antigen on Hodgkin's disease cells, and presently is widely used as a clinical marker for a number of hematologic malignancies (for review, see Horie and Watanabe, *Immunol* 10:457-470 (1998)). A naturally-occurring soluble form of the CD30 protein is found in human serum, and levels of this protein are elevated in a variety of pathological conditions including viral infection, allergic and autoimmune conditions and neoplastic diseases.

CD30 and CD30L are expressed on T cells, and appear to be involved in regulation of the immune system. Their expression on T cells is activation-dependent. CD30 has been reported to be a specific marker of T_H2 type T cells (Romagnani, *J Leukocyte Biol* 57:726 (1995); Romagnani, *J Clin Immunol* 15:121-129 (1995)). The T_H1 and T_H2 subsets of $CD4^+$ T cells can be distinguished based on which cytokines they predominantly express. Though individual T cells may actually secrete mixtures of these two groups of cytokines, chronic immune reactions are often dominated by one type or the other of $CD4^+$ T cells. T cells that produce T_H2 cytokines, which include IL-4, generally are considered to be mediators of allergic reactions. It has been suggested that the detection of circulating $CD30^+$ T cells could serve as a marker for T_H2-dominated

allergic conditions such as atopic dermatitis (Yamamoto et al., *Allergy* 55:1011-18 (2000)); however, the correlation between CD30 expression and T_H2 phenotype has not held up over time (see, for example, Bengtsson et al, *J Leukocyte Biol* 58:683 (1996); Hamann et al., *J Immunol* 156:1387-91 (1996)). Based on experiments using a mouse model of diabetes, it has been proposed also that CD30-mediated signaling may protect against autoimmune disease (Kurts et al., *Nature* 398:341-344 (1999)). Others have reported that IL-4 upregulates, whereas IFNγ downregulates, the expression of CD30 on activated T cells (Nakamura et al., *J Immunol* 158:2090-98 (1997); Gilfillan et al., *J Immunol* 160:2180-87 (1998)).

The naturally-occurring ligand for CD30, CD30L, is a type II membrane glycoprotein that binds specifically with CD30, thus triggering CD30 to transmit a signal via its cytoplasmic domain. The isolation of mouse and human cDNAs encoding CD30L is described in U.S. 5,480,981. In addition to being expresed on activated T cells, CD30L is expressed on monocytes/macrophages, granulocytes and a subset of B cells (see, for example, U.S. 5,480,981). CD30L has been reported to induce murine B cell differentiation and the proliferation of activated T cells in the presence of an anti-CD3 co-stimulus (see, for example, Smith et al., *Cell* 73:1349-1360 (1993)). Moreover, it has been reported that CD30L exhibits "reverse signaling," that is, the cell surface CD30L that is expressed on neutrophils and peripheral blood T cells can be activated by cross-linking to stimulate metabolic activities in those cells (Wiley et al., *J Immunol* 157: 3235-39 (1996)).

There is a need to better understand the biological activities of CD30 and CD30L, and to exploit this knowledge in the treatment of human disease.

SUMMARY OF THE INVENTION

In accord with this invention, an agent capable of inhibiting the binding of CD30 to CD30L is used for treating an autoimmune or chronic inflammatory condition, the method comprising administering the agent to the patient according to a regimen of dose and frequency of administration that is adequate to induce a sustained improvement in at least one indicator that reflects the severity of the patient's condition. The improvement is considered to be sustained if the patient exhibits the improvement on at least two occasions separated by at least one day. The agent may be formulated into a physiologically acceptable pharmaceutical preparation, which may be packaged with a written matter describing the foregoing use. Moreover, an inhibitor of the CD30/CD30L

interaction according to this invention may be administered concurrently with other treatments being used to treat the same disorder. In a preferred embodiment, the patient is a human.

In one of the embodiments of the invention, preferred agents for use in treating an autoimmune or inflammatory condition include an antibody that is specific for CD30L, a non-agonistic antibody that is specific for CD30, and a soluble CD30 polypeptide that comprises all or part of the extracellular region of human CD30. The nucleotide and amino acid sequences of human CD30 are shown in SEQ ID NO:6. Suitable CD30 polypeptides for use as therapeutic agents include proteins that comprise amino acids 19-390 of SEQ ID NO:6, or a fragment thereof that retains the ability to bind CD30L, including polypeptides having an at least 80%, at least 85%, at least 90%, at least 95%, at least 97.5%, or at least 99%, and most preferably at least 99.5% sequence identity with amino acids 19-390 of SEQ ID NO:6. Such polypeptides if administered *in vivo* are expected to bind with endogenous CD30L thereby interfering with the interaction between endogenous CD30 and endogenous CD30L. Such polypeptides may if desired be conjugated with another moiety, usually another protein, that promotes oligomerization. A moiety suitable for this purpose is the Fc region from an immunoglobulin molecule. Agents that antagonize the CD30/CD30L interaction may be used to prepare a pharmaceutical preparation to be administered in accord with the methods of the invention, either alone or concurrently with other treatments. Such pharmaceutical preparations may be packaged with a written matter describing the aforedescribed uses.

The hereindescribed therapeutic agents that inhibit the CD30/CD30L interaction may be used to treat a variety of diseases, including various arthritic conditions. For example, rheumatoid arthritis may be treated with the CD30/CD30L antagonists that are described above.

In one aspect of the invention, an agent capable of inhibiting the interaction of CD30 and CD30L is used concurrently with a second agent that is capable of antagonizing TNFα, IL-1α, IL-1β or IL-4. Medical disorders expected to be especially responsive to these combination treatments include multiple sclerosis, systemic sclerosis, acute inflammatory demyelinating polyneuropathy, acute motor axonal neuropathy, acute motor sensory axonal neuropathy, Fisher syndrome and systemic lupus erythematosus. As an example, the foregoing medical disorders may be treated with an antibody specific

for CD30L used together with an antagonist of IL-4. The anti-CD30L antibody and the IL-4 antagonist are used for formulating pharmaceutical preparations for this purpose, and may be packaged separately or together in one package with a written matter describing this use. Suitable IL-4 antagonists for use in this method of treatment include but are not limited to an antibody specific for IL-4, an antibody specific for IL-4R and a soluble IL-4 receptor comprising amino acids 1-207 or 2-207 of SEQ ID NO:16. In one of the preferred embodiments of the invention, a patient who is suffering from systemic lupus erythematosus, scleroderma or pemphigus vulgaris is treated concurrently with an inhibitor of the CD30/CD30L interaction and an antibody specific for IL-4, an antibody specific for IL-4R or a soluble IL-4 receptor, wherein the receptor comprises amino acids 1-207 or amino acids 2-207 of SEQ ID NO:16.

In other aspects of the invention, provided are compounds, pharmaceutical preparations and methods of treatment for treating an autoimmune or chronic inflammatory condition that is resistant to treatment with an inhibitor of TNFα. This method comprises administering to a patient in need thereof an agent that is capable of inhibiting the binding of CD30 to CD30L or the binding of IL-1α or IL-1β to IL-R1, thereby blocking signal transduction by CD30 or IL-1. The agent is administered according to a regimen of dose and frequency of administration that is adequate to induce a sustained improvement in at least one indicator that reflects the severity of the patient's condition, the improvement being considered sustained if the patient exhibits the improvement on at least two occasions separated by at least one day. Agents suitable for use in such methods include an antibody that is specific for CD30, CD30L, IL-1α, IL-1β or IL-1R1, wherein the antibody may be a polyclonal antibody, a monoclonal antibody, a humanized antibody or a human antibody. Agents to be used for this purpose may be formulated into pharmaceutical preparations, which may be packaged with a written matter describing such use. One exemplary agent for this purpose is a soluble fragment of IL-1R2 that includes amino acids 1-333 of SEQ ID NO:8, or a subportion thereof that is capable of binding with IL-1α or IL-1β, thereby blocking signal transduction by IL-1α or IL-1β. Another agent suitable for treating such diseases is a soluble CD30 polypeptide that binds CD30L, such as a polypeptide comprising amino acids 19-390 of SEQ ID NO:6 or a CD30L-binding fragment thereof.

In yet another aspect of the invention, provided also is an animal model for screening therapeutic agents, the animal model being characterized by carrying genetic

modifications that inactivate its p55 and p75 TNFα receptor proteins, and also by being genetically susceptible to experimentally-induced arthritis. In a preferred embodiment, the animal model is genetically susceptible is collagen-induced arthritis. For example, inactivation of the p55 and p75 TNFα proteins can be introduced into wild-type DBA/1, BUB or B10.Q mice or into DA, BB-DR or LEW rats, all of which are susceptible to collagen-induced arthritis. In a preferred embodiment, the animal is a DBA/1 mouse that has been genetically modified so that it has double-null mutations in both its p55 and p75 TNFα receptor genes.

The invention also provides methods for using the aforedescribed animal model to screen a candidate therapeutic agent to determine its efficacy in treating an autoimmune or chronic inflammatory condition that is resistant to treatment with a TNFα inhibitor. This method comprises assays in which one induces arthritis in an animal in which the p55 and p75 TNF receptors have been inactivated, administers the candidate therapeutic agent to the animal, and determines that the agent is efficacious if the severity of the animal's arthritis is reduced after the candidate agent has been administered. In a preferred embodiment, the screening assays employ a strain of mouse or rat that is susceptible to collagen-induced arthritis and in which the p55 and p75 proteins have been inactivated by genetic modification. In such mice, arthritis is induced by injecting collagen, the candidate therapeutic agent is administered, and then the severity of the arthritis is assessed by observing the amount of erythema and edema in the animals paws. In a preferred embodiment, the screening assays employ a DBA/1 mouse, a BUB mouse, a B10.Q mouse, a DA rat, a BB-DR rat or a LEW rat. A particularly suitable animal for use in these assays is a DBA/1 mouse carrying double-null mutations in its p55 and p75 TNFα receptor genes.

DETAILED DESCRIPTION OF THE INVENTION

The instant invention discloses methods and compounds for treating or preventing autoimmune and chronic inflammatory diseases, including conditions resistant to treatment with TNFα inhibitors, as well as a model system for screening agents for their efficacy in treating autoimmune or inflammatory conditions that are refractory to treatment with TNFα inhibitors. Patients treated in accord with the invention include those whose condition is continuous or intermittent. Diseases treatable by the subject

methods include, for example, diseases such as arthritis, systemic lupus erythematosus and degenerative conditions of the nervous system, such as multiple sclerosis.

Preferably, the patient undergoing treatment is a mammal, and more preferably is a human. The subject methods are applicable to domestic animals, including pets and farm animals. Provided also herein are methods for using inhibitors that block CD30 or IL-1 signal transduction to treat autoimmune or chronic inflammatory diseases that are resistant to treatment with inhibitors of TNFα. In addition, methods are provided that involve treating the autoimmune or chronic inflammatory diseases described herein concurrently with two or more inhibitors selected from an IL-4 inhibitor, a TNFα inhibitor and an inhibitor of the CD30/CD30L interaction. For purposes of this disclosure, the terms "illness," "disease," "medical condition," "abnormal condition," "malady," "medical disorder", "disorder" and the like are used interchangeably.

Autoimmune conditions are characterized by the production of antibodies or effector T cells that react with native host molecules. Most B cell responses depend on helper T cells, thus the presence of autoantibodies generally involves some dysregulation of T cell function. In some cases, however, autoantibodies arise from normal T and B cell responses to foreign proteins that share antigenic epitopes with the host's own tissues. For example, autoantibodies may be elicited by a pathogenic bacteria that expresses an antigen that resembles a host molecule. In some instances, arthritic syndromes may originate in a woman due to her exposure to fetal cells that have escaped into her circulation during pregnancy. The phrase "chronic inflammatory condition" as used herein refers to chronic disorders whose ongoing symptoms do not appear to be caused by a viral or bacterial infection, even though these diseases in some instances may have been triggered by an infection that no longer is present. Such diseases are "inflammatory" in that they are characterized by the release of inflammatory cytokines such as tumor necrosis factor (TNFα), lymphotoxin α and/or interleukin-1 (IL-1), and they may also involve autoimmunity. In some cases, genetic predispositions play a role in autoimmune or chronic inflammatory diseases that are treatable by the subject methods. For such patients, the subject therapies may be administered prophylactically if desired.

In one aspect of the invention, autoimmune and chronic inflammatory diseases are treated by administering an agent that inhibits signal transduction by CD30. An agent's ability to inhibit CD30 signal transduction can be demonstrated using a biological assay, such as an assay that involves determining that the agent interferes with a biological

activity manifested by $CD30^+$ cells that otherwise would occur when the cells are contacted with CD30L. Alternatively, a CD30 antagonist may be identified by determining its ability to prevent CD30L from binding CD30 that is expressed on the surface of cultured cells. Therapeutic agents of the invention include but are not limited to agents that antagonize the specific binding of CD30 to CD30L. The terms "antagonist" and "inhibitor" are used interchangeably herein.

The term "CD30-ligand" (CD30L) refers to a human or murine CD30-binding protein as disclosed in Smith et al. (1993) and U.S. Patent No. 5,480,981, including CD30-binding muteins thereof. Nucleotide and amino acid sequences for human CD30L are shown in SEQ ID NOS:1 and 2, and those for mouse CD30L are shown in SEQ ID NOS:3 and 4. The extracellular region of human CD30L corresponds to amino acids 1-215 of SEQ ID NO:2. For forming soluble human CD30L molecules that can bind CD30, polypeptides comprising amino acids 44, 45, 46 or 47 through amino acid 215 of SEQ ID NO:2 may be used. The extracellular region of murine CD30L corresponds to amino acids 1-220 of SEQ ID NO:4. For forming soluble murine CD30L molecules that can bind CD30, polypeptides comprising amino acids 49-220 of SEQ ID NO:4 may be used.

As used herein, the phrase "fragment of CD30L" refers to a portion of a full-length CD30L polypeptide that retains the ability to bind to CD30, or that is capable of eliciting an antibody that binds specifically with a CD30L polypeptide of SEQ ID NO:2 or 4 or a subportion thereof. Such fragments preferably will contain at least a portion of the extracellular region of CD30L.

The term "CD30" as used herein refers to the 595 amino acid human CD30 polypeptide encoded by the nucleotide sequence of SEQ ID NO:5, and whose amino acid sequence is shown in SEQ ID NO:6. The cloning of CD30 is described in Durkop et al. (*Cell* 68:421 (1992)). The extracellular portion of human CD30 corresponds to amino acids 1-390 of SEQ ID NO:6, or if the signal peptide is removed, to amino acids 19-390 of SEQ ID NO:6. The phrase "soluble CD30" (sCD30) refers to soluble molecules that comprise all or part of the extracellular domain of the CD30 protein, and that retain the capacity to bind specifically with CD30L. Soluble CD30 polypeptides of the invention encompass recombinant sCD30 and naturally-occurring sCD30 proteins in highly purified form. If desired, the sCD30 may be linked to polyethylene glycol (i.e., pegylated) to

prolong its half-life in the patient's body, or may be linked to another protein moiety that promotes oligomerization.

As used herein, the phrase "fragment of CD30" refers to a portion of a full-length CD30 polypeptide that retains the ability to bind to CD30L, or that is capable of eliciting an antibody that binds specifically with a CD30 polypeptide having the amino acid sequence SEQ ID NO:6 or subportion thereof or to a portion of full-length CD30 that is capable of transmitting a biological signal such as activation of NF-κB.

Soluble CD30 polypeptides according to the invention also include polypeptides that are at least 60%, or at least 70%, or at least 80%, and most preferably at least 90% of the length of the extracellular region of the human CD30 molecule. as shown in amino acids 1-390 of SEQ ID NO:6. Further included as therapeutic agents are proteins comprising a soluble CD30 polypeptide having at least 80%, at least 85%, at least 90%, at least 95%, at least 97.5%, or at least 99%, and most preferably at least 99.5% sequence identity with amino acids 1-390 of SEQ ID NO:6, where sequence identity is determined by comparing the amino acid sequences of the two polypeptides when aligned so as to maximize overlap and identity while minimizing sequence gaps. The value for percent identity in such comparisons can be determined by visual inspection and mathematical calculation. Alternatively, the percent identity of two amino acid sequences can be determined by comparing sequence information using the GAP computer program, version 6.0 described by Devereux et al. (*Nucl. Acids Res.* 12:387, 1984) and available from the University of Wisconsin Genetics Computer Group (UWGCG). The preferred default parameters to be used for the GAP program in making these comparisons include: (1) a unary comparison matrix (containing a value of 1 for identities and 0 for non-identities) for nucleotides, and the weighted comparison matrix of Gribskov and Burgess, *Nucl. Acids Res. 14*:6745, 1986, as described by Schwartz and Dayhoff, eds., *Atlas of Polypeptide Sequence and Structure*, National Biomedical Research Foundation, pp. 353-358, 1979; (2) a penalty of 3.0 for each gap and an additional 0.10 penalty for each symbol in each gap; and (3) no penalty for end gaps. Other programs used by those skilled in the art of sequence comparison may also be used, such as, for example, the BLASTN program version 2.0.9, available for use *via* the National Library of Medicine website, or the UW-BLAST 2.0 algorithm, using standard default parameter settings described at the blast-wustl website. In addition, the BLAST algorithm uses the BLOSUM62 amino acid scoring matrix, and optional parameters that may be used are as

follows: (A) inclusion of a filter to mask segments of the query sequence that have low compositional complexity (as determined by the SEG program of Wootton & Federhen (Computers and Chemistry, 1993); also see Wootton JC and Federhen S, 1996, Analysis of compositionally biased regions in sequence databases, *Methods Enzymol.* **266**: 554-71) or segments consisting of short-periodicity internal repeats (as determined by the XNU program of Claverie & States (Computers and Chemistry, 1993)), and (B) a statistical significance threshold for reporting matches against database sequences, or E-score (the expected probability of matches being found merely by chance, according to the stochastic model of Karlin and Altschul (1990); if the statistical significance ascribed to a match is greater than this E-score threshold, the match will not be reported.); preferred E-score threshold values are 0.5, or in order of increasing preference, 0.25, 0.1, 0.05, 0.01, 0.001, 0.0001, 1e-5, 1e-10, 1e-15, 1e-20, 1e-25, 1e-30, 1e-40, 1e-50, 1e-75, or 1e-100.

The phrase "CD30/CD30L interaction" as used herein refers to the specific binding of CD30 to CD30L, resulting in signal transduction by CD30. This includes instances in which at least one binding partner is a fragment of either CD30 or CD30L, that is, the term may refer to the binding interaction of a CD30 fragment to CD30L, CD30 to a CD30L fragment, or a CD30 fragment to a CD30L fragment. In addition, a CD30/CD30L interaction can involve an analog of CD30 (such as an allelic variant or mutein) that is capable of binding specifically to CD30L, or may involve an analog of CD30L (such as an allelic variant or mutein) that can bind specifically with CD30. Moreover, a CD30/CD30L interaction can involve either endogenous CD30 or CD30L proteins or may involve recombinant CD30 or CD30L expressed by a cell transfected with a nucleic acid encoding the recombinant protein. Similarly, the phrase "IL-1/IL-1R interaction" refers to the specific binding between IL-1 and IL-1R, including instances where at least one of the binding partners is a fragment of the full-length polypeptide, and including instances where one of the binding partners is an allelic variant or mutein that retains the ability to bind specifically with its binding partner.

The term "IL-1" as used herein includes both IL-1α and IL-1β. IL-1α and IL-1β are two distinct proteins that both are instrumental in inflammation, and both of which bind the same two cell surface receptors. The cell surface receptors to which IL-1α and IL-1β both bind are known as types I and II IL-1 receptors, or "IL-1R1" and "IL-1R2." Signal transduction by IL-1 is mediated primarily by IL-1R1, while IL-1R2 is considered to be a "decoy" receptor whose function is to downregulate IL-1 biological activity.

Human IL-1R2 is described in U.S. Patent No. 5,350,683. A nucleic acid sequence encoding the IL-1R2 protein is shown in SEQ ID NO:7, and the amino acid sequence is shown in SEQ ID NO:8.

In one embodiment, the subject invention provides methods of treating autoimmune or chronic inflammatory conditions that are resistant to treatment with drugs that inhibit TNFα. In such patients, the lack of response to TNFα inhibition may be partial or complete. TNFα inhibitors to which these diseases are unresponsive may include one or more of the following: etanercept (p75 TNFR:Fc, sold as ENBREL®; Immunex Corporation); LENERCEPT® (p55 TNFR-Ig fusion protein; Roche); or antibodies against TNFα, including humanized antibodies such as infliximab (REMICADE®; Centocor), D2E7 (BASF Pharma) or CDP571 (HUMICADE®; Celltech). Arthritis that is resistant to one of these or to another TNF inhibitor is referred to as "TNFα-independent arthritis." Diseases resistant to treatment with TNFα inhibitors may be treated by administering one or more agents that inhibit signal transduction by CD30 or IL-1, such as an inhibitor of the CD30/CD30L interaction and/or of the IL-1/IL-1R interaction. In addition, diseases that are less than fully responsive to TNFα inhibitors are treated with a combination of a TNFα inhibitor administered concurrently with an inhibitor of the CD30/CD30L interaction and/or with an inhibitor of an IL-1/IL-1R interaction.

Therapeutic agents

Any physiologically acceptable agent capable of blocking signal transduction by CD30 may be used as a therapeutic agent in the disclosed methods of treatment, including but not limited to: antibodies that bind specifically to CD30L and thereby inhibit its binding to CD30; non-agonistic antibodies that bind specifically to CD30 and inhibit its ability to transmit a biological signal; muteins or analogs such as allelic variants of CD30L or fragments thereof that bind to CD30 but that do not stimulate transduction of a biological signal; sCD30 molecules that contain the extracellular domain of CD30 or a portion thereof that is capable of binding CD30L; and small organic molecules that block signal transduction by CD30 or that interfere with the biological functioning of CD30L.

Soluble CD30 molecules used as therapeutic agents as described herein comprise the extracellular region of CD30. An exemplary CD30 protein is shwon in SEQ ID NO:6, in which amino acids 1-390 of SEQ ID NO:6 correspond to the extracellular region. If less than the entire extracellular domain is used, the fragment must retain the

ability to bind CD30L, which ability may be ascertained using any convenient binding assay format. The presence of the signal peptide in the sCD30 is optional. Suitable assays include testing for the ability of the sCD30 to competitively block binding of labeled CD30L to cells expressing surface CD30 or to block binding of isolated CD30L or cells expressing surface CD30L to cell-surface CD30 or CD30 that is anchored to a solid support such as an ELISA plate or a chromatography matrix, such as agarose beads. When used therapeutically, these sCD30s block the binding of membrane-bound CD30 to cells expressing CD30L

sCD30s useful as CD30 antagonists for the disclosed therapeutic methods include oligomeric sCD30 polypeptides (such as dimers or trimers), as well as monomers. Oligomers may be linked by disulfide bonds formed between cysteine residues on different sCD30 polypeptides. In one embodiment of the invention, the therapeutic agent is a sCD30 that is created by fusing the extracellular domain of CD30 (or a CD30L binding portion thereof) to the Fc region of an antibody, preferably a human antibody, in a manner that does not interfere with binding of the CD30 moiety to CD30L. The resulting fusion protein is a CD30:Fc that will dimerize by the spontaneous formation of disulfide bonds between the Fc moieties on two of the fusion protein chains. Native Fc region polypeptides or muteins thereof may be employed in making these constructs. Suitable CD30:Fc's will reduce or abolish the ability of agonistic anti-CD30 antibodies to stimulate CD30, or will competitively inhibit the binding of CD30L to membrane-bound CD30. An example of a suitable CD30:Fc protein that may be used in the subject methods is one that is constructed as described in U.S. 5,677,430.

Other suitable oligomeric sCD30 polypeptides may be prepared by fusing the extracellular domain of CD30 (or a fragment thereof) to a "leucine zipper," which is a peptide that promotes oligomerization of the proteins in which they are present. Leucine zippers are motifs found in several DNA-binding proteins (see, for example, Landschulz et al., *Science* 240:1759 (1988)), and naturally-occurring leucine zipper peptides and derivatives thereof can promote the formation of dimers or trimers of protein chains in which they are present. Examples of leucine zipper domains useful for producing soluble oligomeric proteins are described in WO 94/10308.

Another method of linking multiple copies of soluble CD30 polypeptides is by fusing monomers together with suitable peptide linkers. A fusion protein comprising two or more copies of sCD30 separated by peptide linkers may be produced by recombinant DNA technology. Such peptide linkers optimally are from 5 to 100 amino acids in

length, preferably comprising amino acids selected from the group consisting of glycine, asparagine, serine, threonine, and alanine. The production of recombinant fusion proteins comprising peptide linkers is illustrated in U.S. 5,073,627.

In yet other embodiments, antagonists can be designed to reduce the level of endogenous CD30 or CD30L gene expression, for example, by using well-known antisense or ribozyme approaches to inhibit or prevent translation of CD30 or CD30L mRNAs; triple helix approaches to inhibit transcription of CD30 or CD30L genes; or targeted homologous recombination to inactivate or "knock out" the CD30 or CD30L genes or their endogenous promoters or enhancer elements. Such antisense, ribozyme, and triple helix antagonists may be designed to reduce or inhibit either unimpaired, or if appropriate, mutant CD30 or CD30L gene activity. Techniques for the production and use of such molecules are well known to those of skill in the art.

Antisense RNA and DNA molecules can act to directly block the translation of mRNA by hybridizing to targeted endogenous mRNA thereby preventing translation. Alternatively, antisense RNA or DNA can inhibit or prevent transcription of the target gene. The antisense approach involves designing oligonucleotides (either DNA or RNA) that are complementary to a CD30 or CD30L mRNA, or complementary to a portion of the target gene, such as a regulatory element that controls transcription of the gene. Antisense nucleic acids should be at least six nucleotides in length, and are preferably oligonucleotides ranging from 6 to about 50 nucleotides in length.

In one embodiment of the invention, ribozyme molecules designed to catalytically cleave CD30 or CD30L mRNA transcripts are used to prevent translation of CD30 or CD30L mRNA and expression of CD30 or CD30L polypeptides. (See, *e.g.*, WO 90/11364, or U.S. Patent No. 5,824,519).

Provided herein are therapies for treating autoimmune and chronic inflammatory conditions that are resistant to treatment with an inhibitor of TNFα. Therapies according to this embodiment of the invention are administered to a patient who has already been observed to be partially or fully unresponsive to treatment with a TNFα inhibitor. To treat a patient whose autoimmune or chronic inflammatory condition is resistant to a TNFα inhibitor, the patient is administered an effective amount of an agent that is capable of blocking the binding of CD30 to CD30L, such as one of the inhibitors described above. Alternatively, the patient is administered an IL-1 antagonist that is capable of inhibiting signal transduction by IL-1, such as by reducing the amount of IL-1 produced in the

patient's body, or by interfering with the binding of IL-1 to IL-1R1. In the case of patients who are partially responsive to treatment with a TNFα inhibitor, treatments according to this embodiment of the invention may be administered concomitantly with a TNFα inhibitor. The therapeutic agents of this embodiment of the invention generally are administered in the form of a physiologically acceptable composition.

In a further embodiment of the invention, patients are treated with an inhibitor of the CD30/CD30L interaction administered concurrently with an antagonist of IL-4. IL-4 is a cytokine with a broad range of activities, and is expressed by $CD30^+$ T cells. The cell surface receptors to which IL-4 binds are referred to as the "IL-4 receptor" or "IL-4R." (see for example, U.S. Patent No. 5,599,905). The interaction between IL-4 and its receptor can be inhibited by administering a soluble IL-4 receptor (sIL-4R), such as the sIL-4Rs described in U.S. Patent No. 5,599,905. Preventing this interaction will hinder or prevent IL-4-mediated biological activities. IL-4 can induce a chronic inflammatory effect in some diseases and can exacerbate some autoimmune disorders. In such diseases, the infiltration and proliferation of T_H2 cells is fueled by IL-4 and CD30 signal transduction. These cells cause the overproduction of IL-4. Accordingly, diseases where this occurs, including atopic dermatitis, asthma, systemic lupus erythematosus, scleroderma or pemphigus vulgaris, are effectively treated by administering an agent that inhibits IL-4 concurrently with an agent that inhibits the interaction between CD30 and CD30L. The latter preferably is an antibody that is specific for CD30L.

Methods of using IL-4 inhibitors to treat immune or inflammatory responses are illustrated, for example, in U.S. Patent No. 5,767,065. IL-4 antagonists that may be administered in combination with inhibitors of the CD30/CD3-L interaction include, but are not limited to, IL-4 receptors (IL-4R) and other IL-4-binding molecules, IL-4 muteins and antibodies that bind specifically with IL-4 or IL-4 receptors thereby blocking signal transduction, as well as antisense oligonucleotides and ribozymes targeted to IL-4 or IL-4R. Polyclonal or monoclonal antibodies specific for IL-4 or IL-4 receptor may be prepared using standard procedures. Among the IL-4 receptors suitable for use as described herein are soluble fragments of human IL-4R that retain the ability to bind IL-4. Such fragments retain all or part of the IL-4R extracellular region and are capable of binding IL-4. In a preferred embodiment of the invention, the patient is treated concurrently with sIL-4R and an antibody specifically for CD30L.

IL-4 receptors are described in U.S. Patent 5,599,905; Idzerda et al., *J. Exp. Med.* 171:861-873, March 1990 (human IL-4R); and Mosley et al., *Cell* 59:335-348, 1989 (murine IL-4R), each of which is hereby incorporated by reference in its entirety. The protein described in those three references is sometimes referred to in the scientific literature as IL-4Rα. Unless otherwise specified, the terms "IL-4R" and "IL-4 receptor" as used herein encompass this protein in various forms that are capable of functioning as IL-4 antagonists, including but not limited to soluble fragments, fusion proteins, oligomers, and variants that are capable of binding IL-4. Suitable IL-4Rs include variants in which valine replaces isoleucine at position 50 (see Idzerda et al., 1990), and include slow-release formulations, and PEGylated derivatives (modified with polyethylene glycol) are contemplated, as well as recombinant fusion proteins comprising heterologous polypeptides fused to the N-terminus or C-terminus of an IL-4R polypeptide, including signal peptides, immunoglobulin Fc regions, poly-His tags or the FLAG® polypeptide described in Hopp et al., *Bio/Technology* 6:1204, 1988, and U.S. Patent 5,011,912, as well as fusions of IL-4 receptors with oligomer-promoting leucine zipper moieties. Soluble recombinant fusion proteins comprising an IL-4R and immunoglobulin constant regions are described, for example, in EP 464,533. A nucleotide sequence encoding human IL-4 receptor is shown in SEQ ID NO:15 and amino acid sequence for human IL-4 receptor is shown in SEQ ID NO:16. In a preferred embodiment, the IL-4 antagonist to be used in combination with an inhibitor of the CD30/CD30L interaction is a soluble human IL-4 receptor comprising amino acids 1 to 207 of SEQ ID NO:16 and in another preferred embodiment, the IL-4 antagonist comprises amino acids 2 to 207 of SEQ ID NO:16. IL-4 antagonists useful for the hereindescribed methods of treatment also include molecules that selectively block the synthesis of endogenous IL-4 or IL-4R, such as antisense nucleic acids or ribozymes targeted against either of these molecules.

Various IL-4 antagonists that may be used for the hereindescribed methods of treatment can be identified, for example, by their ability to inhibit ^{3}H-thymidine incorporation in cells that normally proliferate in response to IL-4, or by their ability to inhibit binding of IL-4 to cells that express IL-4R. In one assay for detecting IL-4 antagonists, one measures the ability of a putative antagonist to block the IL-4-induced enhancement of the expression of CD23 on the surfaces of human B cells. For example, B cells isolated from human peripheral blood are incubated in microtiter wells in the presence of IL-4 and the putative antagonist. Following the incubation, washed cells are

then incubated with labelled monoclonal antibody against CD23 (available from Pharmingen) to determine the level of CD23 expression. An anti-huIL-4R murine mAb (R&D Systems) previously shown to block the binding and function of both hIL-4 and hIL-13 may used as a positive control for neutralization of CD23 induction by IL-4. Alternatively, suitable IL-4 antagonists may be identified by determining their ability to prevent or reduce the impaired the barrier function of epithelium that results when IL-4 is incubated with the epithelium. For this purpose, one may use confluent monolayers of human epithelial cell lines such as Calu-3 (lung) or T84 (intestinal epithelium). Incubation of such monolayers with IL-4 causes significant damage to their barrier function within about 48 hours. To assay IL-4 antagonists, monolayers may be tested for their permeability, for example, by adding radiolabeled mannitol to cells incubated with IL-4 in the presence or absence of an antagonist. Alternatively, transepithelial resistance (indicating an intact barrier) may be determined using a voltmeter.

In other embodiments of the invention, antagonists of the CD30/CD30L interaction are administered concurrently with an IL-1 antagonist. Alternatively, IL-1 antagonists may be administered alone for treating autoimmune or inflammatory conditions that are resistant to treatment with TNFα inhibitors. Suitable agents for inhibiting signal transduction by IL-1 include antibodies specific for IL-1 or IL-1R1, particularly humanized antibodies. Other suitable IL-1 antagonists include: IL-1 receptor antagonist (IL-1ra); receptor-binding fragments of IL-1 that block native IL-1 from binding IL-1R1; antibodies directed against IL-1 or IL-1R1; and recombinant proteins comprising all or part of a receptor for IL-1 or modified variants thereof, including genetically-modified muteins, multimeric forms and sustained-release formulations. IL-1ra is a naturally-occurring endogenous antagonist of IL-1 and binds both IL-1R1 and IL-1Rs. Preferred IL-1 antagonists include soluble IL-1R2 molecules that are capable of binding IL-1 and that comprise all or part of the extracellular domain of IL-1R2. These soluble IL-1R2 molecules block IL-1 from interacting with membrane-bound IL-1R1. Other useful IL-1 antagonists include soluble forms of IL-1R1 that are capable of competitively inhibiting the interaction of IL-1 with IL-1R1 and further include IL-1β converting enzyme (ICE) inhibitors, which generally are small organic molecules. A preferred IL-1 antagonist is a soluble fragment of IL-1R2 that includes amino acids 1-333 of SEQ ID NO:8, or a subportion thereof that is capable of binding specifically with IL-1α or IL-1β. Soluble IL-1R2s to be used in accord with the invention include, for

example, analogs or fragments of native IL-1R2 having at least 20 amino acids, that lack the transmembrane region of the native molecule, and that are capable of binding IL-1. The ability of soluble IL-1R2 to bind IL-1 (including binding to fragments of IL-1α or IL-1β) can be assayed using ELISA or any other convenient assay.

Other suitable IL-1 antagonists are chimeric proteins in which one of the IL-1 antagonists described above is fused with another polypeptide that promotes the spontaneous formation of a dimer, trimer or higher order multimer. A suitable polypeptide moiety for promoting dimerization is the Fc region of a human immunoglobulin. For example, soluble IL-1R2 polypeptides or fragments thereof may be fused the Fc region of an immunoglobulin to form a chimeric protein that is capable of dimerizing. Any of the foregoing IL-1 antagonists, other than ICE inhibitors, may be covalently linked to polyethylene glycol (pegylated) to prolong their half-life in the body.

It is understood that while IL-1α and IL-1β are the IL-1s most commonly associated with inflammation, other forms of IL-1 exist, and the invention encompasses the use of therapeutic agents targeting these other forms for treating autoimmune and chronic inflammatory diseases that are resistant to treatment with TNFα inhibitors.

Preferred agents for use in the subject therapeutic methods include antibodies that block the CD30/CD30L, IL-1/IL-1R or IL-4/IL-4R interactions. Such antibodies are specifically immunoreactive with their target, that is, they bind to the target protein via the antigen-binding site of the antibodies and do not bind unrelated proteins to a significant degree. Antibodies specific for CD30L will bind endogenous CD30L, thus reducing the amount of endogenous CD30L available for binding to cell surface CD30. Also suitable for use as a therapeutic agent of the subject invention are biologically active fragments of antibodies. For example, a biologically active fragment of an anti-CD30L antibody is an antibody protein that is truncated relative to the intact antibody, but that retains the ability to specifically bind CD30L and to block its interaction with CD30. Antigen-binding fragments of antibodies, include, but are not limited to, Fab and $F(ab')_2$ fragments, and may be produced by conventional procedures.

Antibodies that antagonize the CD30, IL-1 or IL-4 signal transduction can be identified in any suitable assay, including assays based on biological function or assays based on the detection of physical binding. Examples of such assays are disclosed, for example, in U.S. 5,677,430. One preferred assay tests an antibody's ability to block the binding of cell surface CD30L to cell surface CD30. Cell lines suitable for such assays

include the CD30+ HDLM-2 or L540 cell lines, or may use activated T cells expressing CD30. An assay based on biological function, for example, could entail determining whether an antibody can antagonize the ability of membrane-bound CD30L to stimulate proliferation of $CD30^+$ cells that are responsive to such stimulation. Yet another assay employs the $CD30^+$ K299 human cell line. It has been observed that proliferation of these cells is inhibited by contact with CD30L-transfected cells. An antibody specific for CD30L (or other antagonist that blocks CD30L) could suppress this effect of CD30L and thus enhance the proliferation of these cells in this assay. In other instances, the antibody is tested for its ability to block the binding of labeled recombinant CD30L to cell surface CD30. In other instances, the assays employ cells transfected with DNA encoding human CD30L, IL-1R1, IL-1R2 or IL-4R. For example, the ability of a monoclonal antibody against human CD30L to block CD30/CD30L interactions can be assessed by determining whether it can block the binding of human CD30:Fc to either cells transfected with human CD30L or to activated human T cell blasts as assessed by FACS analysis. Similarly, the specificity of an antibody for IL-4R could be tested by determining if the antibody blocks binding of labeled IL-4 to IL-4R.

Other suitable assays utilize soluble forms of both binding partners, for example, sCD30 and sCD30L. For example, sCD30 may be bound to a solid phase, such as a column chromatography matrix or to an ELISA plate. For an ELISA assay using sCD30 and sCD30L, a sCD30, such as CD30:Fc, is fixed to an ELISA plate, and an antibody raised against CD30L is tested to see if it is an antagonist by checking its ability to inhibit the binding of a soluble CD30L leucine zipper construct to the anchored sCD30. In this assay, binding of the leucine zipper construct or other soluble CD30L to the ELISA plate is measured using a biotinylated non-neutralizing anti-CD30L monoclonal antibody, or alternatively, by using an antibody to the leucine zipper end of the CD30L construct. Other assays test the ability of an antibody to block the binding of IL-1 to cell surface IL-1R1.

Therapeutic agents suitable for use in the subject methods include non-agonistic antibodies against human CD30. Such antibodies can block the CD30/CD30L interaction. Monoclonal antibodies against human CD30 can be prepared, for example, as described in U.S. 5,677,430, then tested to determine if they are agonistic or non-agonistic. A non-agonistic antibody against CD30 may be distinguished from an agonistic antibody by testing its effect on CD30 in a suitable biological assay, such as the assays described U.S. 5,677,430. In one such assay, an antibody specific for CD30 is

tested to determine whether it can induce proliferation of activated T cells prepared from peripheral blood or whether it can induce proliferation of the Hodgkin's disease-derived cell lines HDLM-2 or L-540. An agonistic antibody, will induce such proliferation. In contrast, a non-agonistic antibody against CD30 will bind specifically with CD30 but will not induce proliferation of target cells in these or other assays that rely on signal transduction by CD30.

Therapeutic agents according to this invention may be administered concurrently with one or more additional therapeutic molecules to treat autoimmune or chronic inflammatory diseases. As used herein, "concurrently" includes instances where the drugs in a combination treatment are administered over the same time period or are alternated. This includes simultaneous and sequential administration, and the different drugs may be present in the same or separate pharmaceutical compositions. The frequency and route of administration for the different drugs in such combinations may be the same or different. Therapeutic agents that may be used in such combinations include, for example, antagonists of CD30, IL-1 or IL-4 as described above, and also include non-steroidal anti-inflammatory drugs (NSAIDs), corticosteroids, analgesics, cytokine suppressive drugs, disease-modifying anti-rheumatic drugs (DMARDs), methotrexate, and so on. As an example, antagonists of CD30 or IL-1 may be combined with each other or with antagonists of TNFα, IL-2, IL-6, RANK or other cytokines that may contribute to autoimmunity or chronic inflammation. In some preferred embodiments, the additional therapeutic agents target a cytokine. An antagonist that targets a cytokine may comprise a soluble receptor against the cytokine, and usually includes part or all of the extracellular domain of a receptor for the cytokine. The soluble cytokine receptor may be used as a monomer, or as a dimer or higher multimer (for example, as a fusion molecule wherein the soluble receptor is attached to the dimer-promoting Fc portion of human immunoglobulin). In other embodiments, the soluble cytokine receptor is pegylated to increase its serum half-life. In some embodiments, the soluble cytokine antagonist comprises a soluble TNF receptor (type I or II). Small organic molecules that inhibit inflammatory cytokines may also be used in combination with the subject therapeutic agents. More than one antagonist of CD30 signal transduction may be administered concurrently for treating autoimmune or chronic inflammatory diseases, and may be administered alone or together with other drugs that are effective against the same

autoimmune or chronic inflammatory condition or that are being administered to treat a different condition in the same patient.

Combinations used to treat multiple sclerosis include inhibitors of CD30 administered in conjunction with other drugs used to treat this condition, including but not limited to mitoxantrone (NOVANTRONE®; Immunex Corporation), interferon β-1a (AVONEX®; Ares-Sorono Group), interferon β-1b (BETASERON®; Berlex Laboratories, Inc.) and/or glatiramer acetate (COPAXONE®; Teva Pharmaceuticals). IL-4 antagonists may be added to any of the foregoing combination.

For treating various rheumatic conditions, including rheumatoid arthritis, an agent capable of inhibiting CD30 signal transduction may be administered alone or concurrently with inhibitors of TNFα, such as antibodies against TNFα (for example, humanized antibodies such as REMICADE® (Centocor), D2E7 (BASF Pharma), or HUMICADE® (Celltech)); soluble forms of the TNF receptor (such as ENBREL® (Immunex Corporation) or LENERCEPT® (Roche) or pegylated soluble TNF receptors.

Preparation of Therapeutic Antibodies

CD30L polypeptides suitable for use as an immunogen in producing therapeutic anti-CD30L antibodies include but are not limited to full length CD30L (recombinant or prepared from a naturally-occurring source) or immunogenic fragments thereof, particularly fragments containing all or part of the extracellular domain of CD30L. To be effective as an immunogen, a fragment of CD30L need not retain the capacity to bind CD30, but must be large enough to be antigenic. To be antigenic, a peptide generally must contain at least 20 amino acids. The amino acid sequence of full-length human CD30L is shown in SEQ ID NO:2 and mouse CD30L in SEQ ID NO:4; peptides corresponding to at least 20 contiguous amino acids of either of these proteins may be used as an immunogen to prepare therapeutic agents for use as described herein.

CD30 polypeptides suitable for use as immunogens in producing non-agonistic anti-CD30 antibodies include but are not limited to full length CD30 proteins (recombinant or prepared from a naturally-occurring source) or immunogenic fragments thereof, particularly fragments comprising all or part of the extracellular domain as defined by amino acids 1-390 of SEQ ID NO:6. Immunogens for raising anti-CD30 antibodies preferably contain at least 20 contiguous amino acids of the protein shown in SEQ ID NO:6.

IL-1 or IL-1R polypeptides suitable for use as immunogens in producing antagonistic antibodies include but are not limited to full length proteins (recombinant or prepared from a naturally-occurring source) or immunogenic fragments thereof, particularly fragments comprising all or part of the extracellular domain of human IL-1R2 as shown in amino acids 1-333 of SEQ ID NO:8. Preferred immunogens contain at least 20 contiguous amino acids of the protein shown in SEQ ID NO:8.

Immunogens for raising therapeutic antibodies against TNFα, TNFR, IL-4 or IL4-R will consist of an at least 20 amino acid segment of the target protein. Suitable antigens for raising therapeutic antibodies also include any of the aforementioned immunogens fused to another protein, including proteins fused to an N-terminal "flag" (see, for example, Hopp et al., *Bio/Technology* 6:1204 (1988); U.S. Patent No. 5,011,912), or fused to the Fc portion of an immunoglobulin molecule, preferably a human immunoglobulin. Preferred therapeutic agents include both polyclonal and monoclonal antibodies (MABs), either of which may be generated using the above-described polypeptides as immunogens.

Polyclonal antibodies may be generated according to standard protocols using a variety of warm-blooded animals such as horses, cows, rabbits, mice, rats, or various species of fowl. In general, the animal is immunized with CD30L, CD30, IL-1, IL-1R1, TNFα, TNFR1 or TNFR2, or an immunogen derived therefrom, through intraperitoneal, intramuscular, or subcutaneous injections. The immunogenicity of an immunogenic polypeptide usually is increased through the co-administration of an adjuvant such as RIBI® (Corixa), or Freund's complete or incomplete adjuvant, or other suitable adjuvant. After several booster immunizations, serum samples are collected and tested for specific reactivity with the target polypeptide by any suitable method, such as ELISA, antibody-capture ("ABC") or modified ABC assays, or a dot blot assay. Once the titer of antibody has reached a plateau in terms of reactivity to the target, the polyclonal antiserum is harvested.

For an ABC assay, a plastic dish, such as an ELISA plate, is coated with an antibody that is specifically reactive with the Fc portion of immunoglobulin from for the same species of animal that was used to raise the antibody against the target polypeptide. For example, if a target protein is was injected into a rabbit and the resulting anti-target polyclonal antibody is to be evaluated for specificity, the plates are coated with antibody specifically reactive with the Fc portion of rabbit IgG. In the next step, a sample of

antibody from the immunized rabbit is incubated in the dish under conditions that promote binding between the rabbit IgG and the anchored anti-rabbit antibody. Next, labeled target protein is added, and the dish is incubated under conditions that promote antibody binding. If the sample of antibody being tested is specific for the target protein, then the labeled target protein will become bound to the captured rabbit antibody, and thereafter can be detected after the plate is washed. For example, if the target protein is labeled with biotin, target protein that has become bound can be detected by using a streptavidin-tagged enzyme that is capable of generating a colored product.

Suitable procedures for generating monoclonal antibodies include several methods described in the art (see, for example, U.S. Patent 4,411,993; Kennett et al. (eds.), *Monoclonal Antibodies, Hybridomas: A New Dimension in Biological Analyses*, Plenum Press, New York (1980); and Harlow and Lane (eds.), *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY (1988)). Mice or rats generally are used for the initial immunizations, which are performed as described above for raising polyclonals. After immunization, the immunized animal's spleen cells are harvested and fused according to standard procedures with a myeloma cell line to yield immortalized hybridoma cells that produce monoclonal antibodies. Many myeloma lines suitable for hybridomas are known, including many that are available from the American Type Culture Collection (ATCC), Rockville, Maryland (see *Catalog of Cell Lines & Hybridomas*, ATCC). Individual hybridoma cells are isolated after the fusion step to be screened to identify those producing monoclonal antibodies having the desired specific immunoreactivity. For therapeutic use, high affinity antibodies are preferred. Hybridomas with the desired specification are identified by assays such as ELISA, ABC, modified ABC, or dot blot assays, then are isolated and propagated. The monoclonal antibodies are harvested and purified using standard methods.

Monoclonal antibodies against CD30L may be raised and screened for specific reactivity according to the methods described in U.S. 5,677,430 or by any of the various methods known in the art for raising monoclonals. Screening may involve determining the capacity of the monoclonals to antagonize the binding of cell-surface CD30L to cell-surface CD30 or to antagonize signal transduction by cell-surface CD30.

Antibodies useful for the subject therapeutic methods also include chimeric antibodies and antibodies produced or modified *via* protein engineering or recombinant DNA technology (see, for example, Alting-Mees et al., *Strategies in Molecular Biology* 3:1 (January, 1990)). Production of chimeric and otherwise engineered antibodies is

described in the prior art (see, for example, Reichmann et al., *Nature* 332:323 (1988); Liu et al., *PNAS* 84:3439 (1987); Larrick et al., *Bio/Technology* 7:934 (1989); and Winter and Harris, *TIPS* 14:139 (1993)).

When an antibody is to be administered as a therapeutic agent to a human patient in accord with the subject methods, a humanized antibody is preferred. Even more preferred are human antibodies. Humanized antibodies comprise an antigen binding domain derived from a non-human antibody (for example, a rat or mouse), and may contain the entire variable region of the non-human antibody, or may contain only that portion of the non-human variable region that includes the antigen-binding site. In a preferred embodiment of the invention, the only non-human portion of the humanized antibody is the hypervariable region. Procedures for preparing humanized antibodies are known, and include techniques that involve recombinant DNA technology. Production of humanized antibodies is described, for example, in Winter and Harris, 1993. To create a humanized antibody, DNA encoding the antigen-binding sites of a monoclonal antibody directed against the target may be isolated and inserted directly into the genome of a cell that is producing human antibodies (see Reichmann et al. (1988)). For example, this method could be used for antibodies against CD30, CD30L, IL-1, IL-1R1, TNFα, TNFR-1 or TNRF-2, IL-4 or IL-4R.

In one procedure for preparing humanized antibodies, cDNA is prepared on a mRNA template derived from a hybridoma cell line that produces a non-human monoclonal antibody having the desired specificity. In essence, a fragment of the cDNA that encodes the variable region of the monoclonal antibody (or a fragment thereof containing the antigen binding site) is isolated, and this cDNA fragment is fused to DNA encoding the human counterpart of the remainder of the human antibody molecule, thereby reconstructing a functional antibody molecule. Host cells are transfected with an expression vector containing the fused gene, and are cultured to produce the desired recombinant fusion protein. To isolate DNA encoding the variable region of an immunoglobulin chain, one may employ, for example, the method of Larrick et al. (1989), which involves the polymerase chain reaction (PCR), using a mixture of upstream primers corresponding to the leader sequence, and a downstream primer based on the conserved sequence of the constant region. PCR primers for amplifying the variable region from mouse or human hybridoma cell immunoglobulin mRNA are commercially available (for example, from Stratacyte, La Jolla, California). PCR primers may be used to amplify heavy or light chain variable region DNA, and the resulting amplified DNA

may be inserted into vectors such as ImmunoZAP* H or ImmunoZAP* L (Stratacyte), respectively, for expression in *E. coli.*

To produce a humanized antibody for use as a therapeutic agent in accord with the invention, variable region DNA is isolated from a murine or rat hybridoma cell that expresses an antibody with the desired specificity, and this DNA is fused with a constant region DNA amplified from a cDNA encoding a human antibody. These or similar techniques may be used, for example, to produce a single-chain antigen-binding protein containing a V_L domain fused to a V_H domain through a peptide linker (see Bird et al., *Science* 242:423 (1988)). Further genetic manipulations may be performed to replace all but the hypervariable regions of the antibody with human sequences.

Human antibodies may be generated using methods involving non-human animals. For example, DNA encoding one or more entire human immunoglobulin chains may be introduced into a mouse to produce a transgenic animal, and then the antibody is isolated from cultured cells derived from the mice. The endogenous immunoglobulin genes in the recipient mouse may be inactivated by various means, and human immunoglobulin genes introduced into the mouse to replace the inactivated genes. These genetic manipulations will result in human immunoglobulin polypeptide chains replacing endogenous immunoglobulin chains in at least some instances, and in such mice some or most of the antibodies produced upon immunization will be human antibodies. Examples of techniques for the production and use of such transgenic animals are described in U.S. Patent Nos. 5,814,318, 5,569,825, and 5,545,806.

Animal Model for Diseases Resistant to Treatment with TNFα Inhibitors

TNF inhibition has demonstrated beneficial effects in a significant percentage of rheumatoid arthritis patients and patients with other kinds of arthritis or other chronic inflammatory diseases. However, a subset of rheumatoid arthritis patients respond poorly or not at all to treatment with TNF inhibitors (TNF-independent rheumatoid arthritis). To provide a tool for identifying effective treatments for patients with autoimmune or chronic inflammatory conditions that show little or no improvement in response to treatment with TNFα inhibitors, provided herein is an animal model that is useful for screening candidate therapeutic agents that may be effective for such diseases. The model animal is characterized by carrying genetic modifications that inactivate its p55 and p75 TNFα receptor proteins and by being genetically susceptible to experimentally-induced arthritis. The animal model is created by genetically modifying a strain of

animal that already is known to be genetically susceptible to experimentally-induced arthritis. Provided also are methods for screening candidate therapeutic agents to determine their effectiveness in treating a medical disorder that is resistant to treatment with a TNFα inhibitor. Such medical disorders include rheumatoid arthritis and other kinds of arthritis.

The subject animal model is created by introducing genetic modifications into a strain of animal, usually a mammal, that prior to modification is genetically susceptible to experimentally-induced arthritis. The genetic modifications result in the inactivation of the animal's p55 and p75 TNFα receptor (TNFR) proteins. The p55 and p75 TNFRs are also called the types I and II TNFRs, respectively.

A model animal according to the subject invention may be deficient in its p55 and p75 TNFR proteins as the result of any of several different types of mutation strategies. For example, the deficiency may result from mutations that inhibit the transcription of a translatable TNFR messenger RNA or that result in production of defective TNFR proteins that do not bind TNFα. Cells from the subject animal model will bind no detectable TNFα as compared with animals that express at least one of these TNFR proteins in wild-type form. The ability of cells from a subject animal model to bind TNFα may be assayed, for example, as described in Peschon et al. (1998) or by other suitable methods. For example, cells taken from a genetically modified animal may be tested for expression of functional TNFR I and II by being incubated with labeled biotinylated TNFα and streptavidin-conjugated phycoerythrin, then analyzed by flow cytometry to determine if the phycoerythrin was captured on the cells. Various cells may be isolated from the test animal to be used in such a binding assay, including conconavalin A-stimulated thymocytes, thioglycolate-elicited peritoneal exudate cells and bronchoalveolar lavage cells collected after intranasal administration of lipopolysaccharide (Peschon et al., 1998). If such cells from the mutated animal fail to bind labeled TNFα, this indicates that both types I and II TNFR are suitably inactivated.

In a preferred embodiment of the invention, the animal in which the p55 and p75 proteins are inactivated is a rodent strain that is susceptible to experimental collagen-induced arthritis (CIA). In a preferred embodiment, mutations are introduced into this rodent's genome that result in inactivation of the genes encoding the p55 and p75 TNFα receptors. In one preferred embodiment, the rodent is a strain of mouse or rat. Mice susceptible to CIA include mice that carry the H-2^q MHC haplotype or the H-2^r MHC

haplotype. Exemplary strains of CIA-susceptible mice include the DBA/1, BUB and B10.Q strains, and exemplary strains of CIA-susceptible rats include the DA, BB-DR and LEW strains (see, for example, Joe and Wilder, *Mol Med Today* 5:367-369 (1999) and Anthony and Haqqi, *Clin Exp Rheumatol* 17:240-244 (1999)).

An exemplary animal model according to the invention is a DBA/1 mouse carrying double-null mutations in its p55 and p75 TNFR genes, that is, a $p55^{-/-}p75^{-/-}$ DBA/1 mouse. As used herein, a "null" mutation means that the gene is sufficiently changed relative to the wild-type gene such that it does not give rise to a protein that is recognizable by antibodies specific for the corresponding wild-type receptor protein. This may be accomplished by introducing a deletion or insertion into the wild-type gene, or by other means. Once established in a strain of mouse, a null mutation may be transferred to a different strain by appropriate genetic manipulations. A double-null mutation means that both alleles of that gene carry a null mutation.

In other embodiments, the genetic modifications may be introduced into rodent strains that are susceptible to forms of arthritis other than CIA, such as, for example, one of the arthritis-susceptible strains that are described in Joe and Wilder (1999).

Provided herein are methods that employ the subject animal model for screening assays to determine whether a candidate therapeutic agent is effective for treating autoimmune or chronic inflammatory conditions that are resistant to treatment with a TNFα inhibitor. TNFα inhibitors to which these diseases are unresponsive may include receptor-based TNFα inhibitors such as ENBREL® (Immunex Corporation) and LENERCEPT® (Roche) or other drugs that incorporate a soluble TNFR, humanized antibodies against TNFα such as REMICADE® (Centocor), D2E7 (BASF Pharma) or CDP571 (HUMICADE®; Celltech) or small molecules whose pharmacologic effectiveness may be based on reducing endogenous TNFα (such as, for example, pentoxifylline, thalidomide or others).

Candidate therapeutic agents that are tested in the subject assays are determined to be effective if when administered to the subject animal model the agent brings about a reduction in the severity of arthritis that has been induced in the animal. The severity of arthritis in the animal may be assessed by any desired method, which may be based, for example, on assigning to each animal a numerical score that reflects the degree of swelling or stiffness of the animal's limbs. Efficacy of a test agent in reducing the severity of disease is determined by comparing this score averaged over a group of

arthritic animals that receive the test agent with the score for a group that receives a placebo. The test agent and placebo generally are administered over a period of at least one week, but may be administered for a longer period, for example, over a period of 2, 3, 4, 5, 6, 7 or 8 or more weeks. Alternatively, the effects of a single dose may be assessed using this model.

If DBA/1 $p55^{-/-}p75^{-/-}$ mice with CIA are used in the assays, an efficacious therapeutic agent will partially or fully ameliorate the symptoms of CIA in the mice. A reduction in severity of arthritis is determined to be present in DBA/1 $p55^{-/-}p75^{-/-}$ mice if the average clinical score (determined as described below) for an agent-treated group of mice is one or more clinical score units lower than the average clinical score for a group of negative control mice that are given a placebo. Preferably, the average clinical score in the agent-treated DBA/1 $p55^{-/-}p75^{-/-}$ mice will be at least 2 clinical score units lower than the placebo-treated mice, and more preferably it will be at least 5 units lower.

To determine clinical score for individual DBA/1 $p55^{-/-}p75^{-/-}$ mice in which CIA has been induced, the following index may be used in which each paw is assigned a score in clinical units based on the following:

0 = normal appearance

1 = erythema/edema in 1-2 digits

2 = erythema/edema in >2 digits, or mild swelling in ankle/wrist joint

3 = erythema/edema in entire paw

4 = massive erythema/edema of entire paw extending into proximal joints; ankylosis, loss of function

Paw scores are combined for each mouse to determine a final score for that mouse, and then final scores for all animals in the test agent group are averaged and compared with the average score for the placebo-treated group. Generally, each test group will contain between 5 and 30 animals, though smaller or larger numbers of animals may be used. This or similar clinical scoring systems are suitable for evaluating CIA in DBA/1 $p55^{-/-}p75^{-/-}$ mice, but also are suitable for other species and for animal models involving types of experimental arthritis other than CIA. If desired, other kinds of arthritis scoring systems may be used, such as, for example, a system based on levels in the blood or other tissues of molecules that reflect the degree of inflammation or molecules that are specific disease markers.

When DBA/1 $p55^{-/-}p75^{-/-}$ mice are used, the CIA is induced according to the procedure given in Example 2, or using a similar protocol. In one preferred screening

assay, a test agent is administered to a DBA/1 $p55^{-/-}p75^{-/-}$ mouse that is subjected to CIA induction, with the first dose of the agent being administered on the day the collagen boost is given, or the first dose may be delayed until the onset of symptoms, which generally take 15-60 days to appear in these mice. In this assay, negative control DBA/1 $p55^{-/-}p75^{-/-}$ mice are treated with a placebo, such as rat or mouse IgG or a physiologically acceptable saline solution. If desired, an agent known to be efficacious against CIA in these mice can be included in the assay as a positive control; for example, antibody against CD30L or against Il-1R1 can be used as a positive control (see Example 4), although this is optional. Doses of the test agent and control agent(s) may be administered daily, every two days, every three days, two times per week, one time per week, or less often if desired. The duration of the testing period is variable, and may continue, for example, for three days, one week, two weeks, three or for four or more weeks.

In the subject assays, the test agent and placebo may be administered by any suitable route, including orally administered liquid or solid forms, topical application, aerosol inhalation, transfection of host cells by recombinant DNA expressing the test agent, or by injection, including intraperitoneal, intravenous, subcutaneous or intramuscular injection. If desired, the test agent may be administered via a slow-release implant. When the test agent is incorporated into a slow-release formulation, very few doses are required, and a single dose may be used. If the test agent is an antibody and the subject is a mouse, the candidate therapeutic test agent may be administered at a dose of 20-75 μg/mouse, and is administered every day, every two to four days, or once a week. If the test agent is a small molecule, such as an organic molecule, suitable doses for testing are from 0.5-1000 μg/mouse, and the agent may be administered once every one to ten days. If a larger species of model animal is used, dose is adjusted upward in proportion to average body weight for the larger species.

Efficacy of the test agent in DBA/1 $p55^{-/-}/p75^{-/-}$ mice or another strain of animal is determined by comparing the percentage of animals affected with arthritis in test and negative control groups, or by comparing the percentage of animals exhibiting severe disease during the testing period, and/or by comparing the mean clinical score for control groups and test groups after administration of the test agent. Mean clinical score may be determined according to the index described in Example 2, or by other suitable means. A test agent is determined to be useful for treating disorders resistant to TNFα inhibitors if

animals to whom the test agent is administered exhibit a reduced severity of disease as compared with control animals that receive placebo instead of the test agent. In one preferred embodiment, the observed improvement is statistically significant, that is, $p<0.05$. Optionally, a dose dependency of the therapeutic response is established by administering the test agent at several different doses.

Candidate therapeutic agents to be screened in the subject animal model include any agent that is potentially effective for treating diseases that are resistant to treatment with TNFα inhibitors. This includes, for example, agents that target cytokines other than TNFα that are sometimes associated with inflammation (such as, for example, GM-CSF, interferon-γ, lymphotoxin-α, IL-1, IL-4, IL-8, IL-15, IL-17 and IL-18). Candidate therapeutic agents include soluble receptor molecules and antibodies specific for target cytokines or their receptors. Small organic molecules may also be screened using the subject animal model, including but not limited to agents with potential for interfering with CD30 or IL-1 signal transduction

Therapeutic methods

Disclosed herein are methods for treating a variety of autoimmune and chronic inflammatory diseases by administering to a patient in need thereof an effective amount of one of the above-described agents. Blockers of CD30 signal transduction are used for treating any of the medical disorders listed below, and blockers of CD30 or IL-1 signal transduction are used for treating disorders listed below that respond poorly or not at all to treatment with a TNFα antagonist. In some instances, a disease is generally responsive to treatment with a TNFα inhibitor, but unresponsive in certain patients. An example of such a disease is rheumatoid arthritis. Rheumatoid arthritis patients who respond poorly or not at all to TNFα inhibitors will particularly benefit from treatment with an antagonist of the CD30/CD30L or IL-1/IL-R1 interaction.

Preferably, the patient is a human, and may be either a child or an adult. In one embodiment of the invention, a condition believed to be T_H2 driven or a condition characterized by high levels of expression of CD30 on activated T cells, is treated by administering a CD30 inhibitor concurrently with an IL-4 inhibitor.

For the subject therapeutic methods, the therapeutic agents preferably are administered in the form of a physiologically acceptable composition comprising a purified recombinant protein in conjunction with physiologically acceptable carriers, excipients and/or diluents. Such carriers are nontoxic to recipients at the dosages and

concentrations employed. Compositions suitable for *in vivo* administration may be formulated according to methods well-known in the art. Components that are commonly employed in such formulations include those described in Remington's Pharmaceutical Sciences, 16th ed., 1980, Mack Publishing Company. Ordinarily, the preparation of such compositions entails combining the therapeutic agent with buffers, antioxidants such as ascorbic acid, low molecular weight polypeptides (such as those having fewer than 10 amino acids), proteins, amino acids, carbohydrates such as glucose, sucrose or dextrins, chelating agents such as EDTA, glutathione and other stabilizers and excipients. Neutral buffered saline or saline mixed with non-specific serum albumin are exemplary appropriate diluents. If desired, the therapeutic agent may be formulated as a lyophilizate using appropriate excipient solutions such as sucrose as a diluent. Appropriate dosages can be determined in standard dosing trials, and may vary according to the chosen route of administration. In accordance with appropriate industry standards, preservatives may also be added, such as benzyl alcohol.

The amount and frequency of administration may vary, depending on such factors as the nature and severity of the indication being treated, the desired response, the duration of treatment, the age, weight and condition of the patient, and so forth. The dose of a therapeutic agent may be adjusted to accommodate various routes of administration, or according to the needs of individual patients as determined by the patient's physician.

Arthritis may be treated by the methods and compositions disclosed herein. As used here, the term "arthritis" refers to chronic inflammatory conditions that primarily affect joints, or the connective tissue surrounding joints, although various body organs may also become affected. Arthritis may be autoimmune or traumatic in origin, or it may be triggered by exposure to a foreign antigen, thereafter leading to a chronic condition that is no longer dependent on the continued presence of the triggering antigen. The term "arthritis," as used herein, includes: arthritis deformans; osteoarthritis; rheumatoid arthritis (adult and juvenile); Lyme disease arthritis; reactive arthritis including Reiter's disease; psoriatic arthritis; arthritis nodosa; seronegative spondylarthropathies, including but not limited to ankylosing spondylitis. The efficacy of anti-CD30L treatment in treating arthritic disease is illustrated in Examples 2 and 4.

The subject inhibitors, compositions and combinations are useful in treating a variety of rheumatic disorders, which are defined herein as any chronic disorder involving painful and often multiple localized inflammations of the joints, muscles, nerves, tendons, skin, eyes, connective tissues or various other organ systems. These include but are not

limited to: arthritis; scleroderma; gout; systemic lupus erythematosus; polymyalgia rheumatica; fibromyalgia; Still's disease; chronic uveitis; disorders resulting in inflammation of the voluntary muscle, including dermatomyositis and polymyositis, including sporadic inclusion body myositis; and inflammatory conditions such as chronic back or neck pain and sciatica. Systemic lupus erythematosus can cause inflammation of the joints, skin, kidneys, heart, lungs, blood vessels and brain. In its advanced forms, systemic lupus erythematosus this condition can result in kidney failure. Treatment with antibody against CD30L appeared to delay the progression of kidney failure in a mouse model for this disease (see Example 6).

Provided also are methods for using the subject inhibitors, compositions or combination therapies to treat various disorders of the endocrine system, including but not limited to: juvenile or maturity onset diabetes (including autoimmune, insulin-dependent types of diabetes; non-insulin dependent types and obesity-mediated diabetes); idiopathic adrenal atrophy; Addison's disease; hypothyroidism; Grave's disease; autoimmune thyroiditis, such as Hashimoto's thyroiditis; and polyglandular autoimmune syndromes (types I and II).

Conditions of the gastrointestinal system also are treatable with the subject inhibitors, compositions or combination therapies, including but not limited to: autoimmune sclerosing cholangitis; coeliac disease; inflammatory bowel diseases, including Crohn's disease and ulcerative colitis; autoimmune pancreatitis, including chronic pancreatitis; idiopathic gastroparesis; and idiopathic ulcers, including gastric and duodenal ulcers.

Included also are methods for using the subject inhibitors, compositions or combination therapies for treating disorders of the genitourinary system, such as autoimmune and idiopathic glomerulonephritis; and chronic idiopathic prostatitis (non-bacterial), including benign prostatic hypertrophy.

Also provided herein are methods for using the subject inhibitors, compositions or combination therapies to treat various hematologic disorders, including but not limited to: anemias and hematologic disorders, including pernicious anemia and aplastic anemia, and Fanconi's aplastic anemia; autoimmune hemolytic anemia; idiopathic thrombocytopenic purpura (ITP); myelodysplastic syndromes (including refractory anemia, refractory anemia with ringed sideroblasts, refractory anemia with excess blasts, refractory anemia with excess blasts in transformation); and autoimmune lymphoproliferative syndrome (ALPS).

In addition, the subject inhibitors, compositions and combination therapies are used to treat hereditary conditions such as Gaucher's disease, Huntington's disease, and muscular dystrophy.

The disclosed inhibitors, compositions and combination therapies are furthermore used to treat conditions that affect the liver such as autoimmune or chronic inflammatory hepatitis that is not due to viral infection.

In addition, the disclosed inhibitors, compositions and combination therapies are used to treat various autoimmune or chronic inflammatory disorders that involve hearing loss. One of these is inner ear or cochlear nerve-associated hearing loss that is thought to result from an autoimmune process, i.e., autoimmune hearing loss. This condition currently is treated with steroids, methotrexate and/or cyclophosphamide, which may be administered concurrently with an inhibitor of the CD30/CD30L interaction.

A number of inflammatory pulmonary disorders also can be treated with the disclosed inhibitors, compositions and combination therapies, including: idiopathic lymphangioleiomyomatosis; chronic obstructive pulmonary disease (COPD) associated with chronic non-infectious bronchitis or with emphysema; and fibrotic lung diseases, such as cystic fibrosis and idiopathic pulmonary fibrosis.

Disorders associated with transplantation also are treatable with the disclosed inhibitors, compositions or combination therapies, including graft-versus-host disease. To prevent or ameliorate graft-versus-host disease, the subject inhibitors may be administered prior to, concomitantly with, or following bone marrow or solid organ transplantation, including transplantation of heart, liver, lung, skin, kidney or other organs.

The subject inhibitors and the disclosed compositions and combination therapies also are useful for treating chronic inflammatory eye diseases, including autoimmune uveitis.

The subject inhibitors and the disclosed compositions and combination therapies also are useful for treating inflammatory disorders that affect the female reproductive system, including: multiple implant failure/infertility; fetal loss syndrome or IV embryo loss (spontaneous abortion); and endometriosis.

Other medical disorders treatable with the disclosed inhibitors, compositions and combination therapies include chronic degenerative diseases of the central nervous system. This includes, for example, diseases associated with demyelination, such as multiple sclerosis, systemic sclerosis and the Guillain-Barre syndromes (including acute

inflammatory demyelinating polyneuropathy, acute motor axonal neuropathy, acute motor sensory axonal neuropathy and Fisher syndrome). In a preferred embodiment, multiple sclerosis is treated with an antagonist of the CD30/CD30L interaction (most preferably an antibody against CD30L) alone or concurrently with a TNFα inhibitor or an IL-4 inhibitor (most preferably sIL-4R). Multiple sclerosis is representative of a chronic, degenerative disease of the central nervous system, which besides the demyelinating conditions also include, for example, amyotrohpic lateral sclerosis (Lou Gehrig's Disease); Bell's palsy; Parkinson's disease and idiopathic chronic neuronal degeneration, all of which may be treated with an agent capable of inhibiting the interaction of CD30 and CD30. The efficacy of anti-CD30L antibody in ameliorating a multiple sclerosis-like disease is illustrated in Example 5.

Other chronic inflammatory conditions treatable with the disclosed inhibitors, compositions and combination therapies include cold agglutinin disease; Behcet's syndrome; Sjogren's syndrome; and idiopathic tenosynovitis, as well as various chronic inflammatory disorders associated with hereditary deficiencies. The subject inhibitors, compositions and combination therapies furthermore are useful for treating Bell's palsy (idiopathic facial paralysis); chronic fatigue syndrome (not associated with ongoing infection); chronic degenerative vertebral disc disease; Gulf war syndrome; and myasthenia gravis, which may be treated concurrently with corticosteroids.

Disorders involving the skin or mucous membranes also are treatable using the subject inhibitors, compositions or combination therapies. These include: acantholytic diseases, including Darier's disease, keratosis follicularis, pemphigus vulgaris and paraneoplastic pemphigus; acne rosacea; alopecia areata; bullous pemphigoid; eczema; erythema, including erythema multiforme and erythema multiforme bullosum (Stevens-Johnson syndrome); inflammatory skin disease; lichen planus; linear IgA bullous disease (chronic bullous dermatosis of childhood); loss of skin elasticity; neutrophilic dermatitis (Sweet's syndrome); pityriasis rubra pilaris; psoriasis; pyoderma gangrenosum; loss of skin elasticity; and toxic epidermal necrolysis.

Other diseases that can be treated with the disclosed compounds, compositions and combination therapies include: autoimmune-associated chronic mucocutaneous candidiasis; allergies; sarcoidosis; multicentric reticulohistiocytosis; Wegener's granulomatosis; arteritis, including giant cell arteritis; vasculitis and chronic autoimmune myocarditis.

To treat a medical disorder using the compounds and compositions provided herein, a therapeutically effective amount of a therapeutic agent according to the invention is administered to a mammal in need thereof. The agent is administered according to a regimen of dose and frequency of administration that is adequate to induce a sustained improvement in at least one indicator that reflects the severity of the disorder. An improvement is considered "sustained" if the patient exhibits the improvement on at least two occasions separated by at least one day, but preferably that are separated by one week, two weeks, three weeks or four or more weeks. The severity of the disorder is determined based on signs or symptoms, or may be determined by questionnaires that are administered to the patient, such as the quality-of-life questionnaires often used by physicians to assess the status of chronic disease conditions.

One or more indicators that reflect the severity of a patient's illness may be assessed for determining whether the frequency and duration of drug treatment is sufficient. The baseline value for a chosen indicator is established by examination of the patient prior to administration of the first dose of the therapeutic agent. Preferably, the baseline examination is done within about 60 days of administering the first dose.

For example, if the condition being treated is an arthritic condition, such as rheumatoid arthritis, psoriatic arthritis, osteoarthritis, one or more indicators for determining sufficiency of treatment may be chosen from among: number of tender, painful or swollen joints; degree of joint pain or tenderness or swelling; patient self-assessment (e.g., quality-of-life questionnaires), and physician assessment. For many arthritic diseases, the patient's self-assessment is a satisfactory indicator. Typical self-assessment questionnaires will reflect a patient's ability to conduct their daily activities, their perception of well-being, their level of pain and so on. The duration of treatment required to induce a measurable improvement for an arthritic or any other type of disease treatable as described herein is typically one to several weeks.

If the condition being treated is multiple sclerosis, suitable indicators for determining sufficiency of treatment include observing an improvement in one or more of the following: bladder and bowel control; fatigue; spasticity; body or hand tremors; muscle weakness; ability to walk; numbness in limbs; ability to concentrate (e.g., performance on a simple memory test); and subjective level of pain. Alternatively, the indicator may consist of the patient's score on a quality of life questionnaire as described above.

If the condition being treated is systemic lupus erythematosus, the indicator for determining sufficiency of treatment may consist of an observed improvement in one of the following: fatigue; fever; ulcers of the mouth and nose; facial rash ("butterfly rash"); photosensitivity (SLE often flares up after exposure to sunlight); pleuritis; pericarditis; Raynaud's phenomenon (reduced circulation to fingers and toes with exposure to cold); kidney function; and white blood cell count (SLE patients often have decreased numbers of white blood cells).

Improvement in a patient's condition is induced by repeatedly administering a dose of a therapeutic agent according to the invention until the patient manifests an improvement over baseline for the chosen indicator or indicators. In treating chronic conditions, a satisfactory degree of improvement usually is obtained after repeatedly administering the agent or agents over a period of at least a month or more, e.g., for one, two, or three months or longer. In some instances, improvement may occur sooner than one month, for example, after three weeks, two weeks, one week, or even after a single dose. A treatment duration of one to six weeks, or even a single dose, may be sufficient for treating occasional flare-ups in patients suffering from chronic conditions that tend to go into remission in-between flare-ups. For persistent conditions, treatment may be continued indefinitely if desired. Evan after a condition has shown improvement, maintenance therapy may be continued indefinitely at the same level or at a reduced dose or frequency of administration. If the dose or frequency of administration has been reduced, it may be resumed at the previous level if the patient's condition should worsen. In addition, treatment with inhibitors of the subject invention may be administered prophylactically to patients who are predisposed to an autoimmune or chronic inflammatory condition.

Any efficacious route of administration may be used to therapeutically administer the subject therapeutic agents, combinations and compositions. If injected, the agents can be administered, for example, via intra-articular, intravenous, intramuscular, intralesional, intraperitoneal or subcutaneous routes. Bolus injection or continuous infusion may be used. Other suitable means of administration include sustained release from microparticles, implants or the like, aerosol inhalation, eyedrops, oral preparations, including pills, syrups, lozenges or chewing gum, and topical preparations such as lotions, gels, sprays, ointments or other suitable techniques.

Alternatively, proteinaceous agents, such as antibodies or antigen-binding fragments thereof, may be administered by implanting cultured cells that express the

protein. In one embodiment, the patient's own cells are induced to produce the therapeutic agent by transfection *in vivo* or *ex vivo* with a DNA that encodes a protein that blocks the CD30/CD30L, IL-1/IL-1R, IL-4/IL-4R or TNF/TNFR interactions. This DNA can be introduced into the patient's cells, for example, by using naked DNA or liposome-encapsulated DNA that encodes the agent, by using calcium-phosphate precipitated DNA, or by other means of transfection. Autologous cells that are transfected *ex vivo* are returned to the patient's body.

Regardless of the route of administration that is chosen, it is understood that the treatment regimens may be adjusted depending on the patient's needs, in accord with general principles of medicine.

When the therapeutic agent is an antibody, such as, for example, an antibody against CD30, CD30L, IL-1, IL-1R1, TNFα, IL-4 or IL-4R, preferred dose ranges for therapeutic or prophylactic purposes in humans include 0.1 to 20 mg/kg, or more preferably, 0.5-10 mg/kg. Another preferred dose range is 0.75 to 7.5 mg/kg, as exemplified in the experiments of Example 2 (adjusted for human body weight according to DeVita et al., eds., *Cancer – Principles & Practice of Oncology*, 4th Ed., J.B. Lippincott, 1993). A larger or smaller amount of antibody per dose may be used to accommodate differences in affinity of the antibody for the antigen. A suitable dose range for CD30:Fc is 0.01-20 mg/kg of body weight, or more preferably, 0.1-10 mg/kg of body weight. For other soluble proteins used as inhibitors in accord with the invention, suitable dose ranges include 2-500 μg/kg, 0.5-10 mg/kg and 10 to 50 mg/kg of body weight. It is understood that the skilled physician will adjust the dose and frequency of administration in accord with the needs of the patient and the nature of the disease being treated.

The following examples are offered by way of illustration, and not by way of limitation. Those skilled in the art will recognize that variations of the invention embodied in the examples can be made, especially in light of the teachings of the various references cited herein.

EXAMPLE 1

Monoclonal Antibodies Directed Against CD30L

A monoclonal antibody directed against murine CD30L was produced in rats as follows. Lewis rats were repeatedly immunized by the intraperitoneal route with 10-20 x 10^6 transfected CHO cells expressing full length murine CD30L. When serum

titers were detected in the rats, they were given an intravenous boost with 10×10^6 transfected CHO cells. After three days, spleen cells from the immunized rats were fused with AG8.653 mouse myeloma cells. When hybridomas were well established in 96-well plates, supernatants from each well were screened by an ELISA assay that employed CHO cells that were transfected with DNA encoding CD30L (CELISA). For the CELISA, CHO cells expressing the recombinant CD30L were adhered to the ELISA plates, and each supernatant was screened for its ability to react with the CD30L expressed on the CHO cells. Hybridoma cells from positive wells were expanded in 48-well plates and screened by CELISA and also by fluorescence-activated cell sorting (FACS) using transfected and non-transfected CHO cells. Hybridoma cells that bound transfected but not non-transfected CHO cells were selected and screened further by FACS against mouse lymphoma cells that naturally express CD30L (EL4 cells). Hybridoma isolates that recognized both recombinant and naturally expressed CD30L were cloned twice by limiting dilution cloning, during which the activity of the supernatants was tracked at each step by CELISA and/or FACS assays. One of these clones, the M15 clone, was chosen for further propagation. In additional experiments, it was determined that the M15 antibody specifically blocked the binding interaction between murine CD30 and CD30L.

EXAMPLE 2

Treatment of collagen-induced arthritis in mice

Arthritis treatments that are effective in treating collagen-induced arthritis (CIA) are also effective in treating arthritis in humans (see, for example, Anthony and Haqqi, *Clin Exp Rheumatol* 17:240-244 (1999)), hence the effects of antagonizing the CD30/CD30L interaction was tested in CIA mice. Collagen-induced arthritis (CIA) was elicited in male DBA/1 mice (Harlan, UK) by injecting the mice with type II chicken collagen (Sigma). Mice were injected on day 0 with 100 μg of the collagen in complete Freund's adjuvant, and boosted on day 21 with a dose of 200 μg in incomplete Freund's adjuvant. Collagen injections were administered intradermally at the base of the tail. In this model, generally 75-100% of the mice are affected, that is, 75-100% of the mice exhibit arthritis symptoms after collagen injection. In these experiments, indicia of CIA usually appeared in affected mice by day 23.

Mice injected with collagen as described above were injected intraperitoneally with doses ranging from 0.15-150 μg of the M15 monoclonal antibody whose preparation

is described in Example 1. Four experiments were conducted, each involving 13-15 mice per test group. In each of the four experiments, positive control mice received 150 μg of etanercept (ENBREL®, Immunex Corporation), which is known to be effective against CIA, and negative control mice received this same amount of human IgG (huIgG) or rat IgG. Daily injections of M15, etanercept or IgG were initiated on day 21, i.e., the day of the second collagen injection, and were continued until day 33.

During these experiments, mice were assessed three times per week for clinical signs of arthritis by an independent observer blinded to the treatment groups. Disease was evaluated using an arthritis index system that has been established for this model system. For scoring, each paw was assigned a clinical score based on the index. Paw scores were combined for each animal to determine a clinical score for that animal. The index used was as follows:

0 = normal appearance

1 = erythema/ edema in 1-2 digits

2 = erythema/ edema in >2 digits, or mild swelling in ankle/wrist joint

3 = erythema/ edema in entire paw

4 = massive erythema/edema of entire paw extending into proximal joints; ankylosis, loss of function

Improved mean clinical scores were evident in all groups that received M15 by the fifth day of M15 administration, and in one experiment by the third day of administration. Final results of the four experiments are summarized in Tables 1-4, shown below. Mean clinical scores, percent disease incidence (mice exhibiting CIA divided by total number of mice in group), and percent of affected mice exhibiting severe disease were calculated for each group of mice. Mice considered to have "severe disease" are those that had a clinical score greater than two at any time during the experiment. Statistical significance was determined for the differences in mean clinical score between the negative control group and the other groups of mice, based on scores on the last day of the experiment. Statistical significance was determined using a one-way analysis-of-variance (ANOVA) with Dunnett's method (H.J. Motuisky, *Analyzing Data with GraphPad Prism*, 1999, GraphPad Software, Inc., San Diego, CA). One-way ANOVA compares three or more groups when the data are categorized in one way. Dunnett's method compares control groups to treatment groups.

The first experiment tested the effects of administering to CIA mice a dose of 150 μg/day of the M15 antibody. By day 4 or 5 of this experiment, mean clinical score differences began to appear between negative control animals and those that received M15 or etanercept. The results of this first experiment are summarized in Table 1. As was expected, the mice that received etanercept (positive control group) exhibited a lower incidence of disease and a reduced incidence of severe disease when compared with the negative control mice that received HuIgG. The mice that received M15 also exhibited a lower disease incidence and a reduced percentage of mice with severe disease (see Table 1) as compared with negative controls. In addition, the M15 group had a lower mean clinical score than the negative controls on the last day of the experiment (day 33). For both the etanercept and the M15 groups, the last day differences in mean clinical score relative to negative controls were found to be statistically significant ($p = 0.05$ or less).

TABLE 1

Treatment	% Disease Incidence	Severe Disease	Mean Clinical Score on Last Day of Experiment
M15	53%	27%	2.53
etanercept	33%	7%	0.53
HuIg	87%	80%	6.47

A second experiment employed a similar protocol to compare doses of 50 μg and 150 μg of M15. The results are shown in Table 2. For this experiment, statistically significant improvement in mean clinical score was seen at the end of the experiment for both the etanercept group and for both doses of M15.

TABLE 2

Treatment	% Disease Incidence	Severe Disease	Mean Clinical Score on Last Day of Experiment
M15, 150 μg	40%	20%	1.2
M15, 50 μg	33%	20%	1.07
etanercept	47%%	6%	0.73
HuIg	87%	80%	7.53

In a third experiment, doses of 15, 50 and 150 μg of M15 were compared, and the results are summarized in Table 3. Again, mice that received M15 experienced a lower incidence of disease, as well as a lower incidence of severe disease as compared with negative controls. A statistically significant improvement in mean clinical score vis-a-vis controls was observed at all three doses of M15 in this experiment.

TABLE 3

Treatment	% Disease Incidence	Severe Disease	Mean Clinical Score on Last Day
M15, 150 μg	42%	42%	3.29
M15, 50 μg	71%	57%	3.71
M15, 15 μg	47%	47%	3.3
etanercept	53%	20%	1.33
HuIg	87%	80%	8.4

In a fourth experiment, 0.15, 1.5, 15 and 150 μg doses of M15 were tested, using rat IgG as the negative control. Results of this experiment are shown in Table 4. As seen in Table 4, improvement in mean clinical score exhibited a dose-dependency at the higher doses. For the 15 and 150 μg doses, the improvement in clinical score was statistically significant.

TABLE 4

Treatment	% Disease Incidence	Severe Disease	Mean Clinical Score on Last Day
M15, 150 μg	33%	26%	1.47
M15, 15 μg	80%	33%	2.67
M15, 1.5 μg	73%	67%	5.47
M15, 0.15 μg	67%	67%	5.67
etanercept	40%	13%	1
rat IgG	87%	67%	5.8

EXAMPLE 3

TNF-independent model of murine collagen-induced arthritis

A novel mouse was generated by moving previously described targeted null mutations in both the p55 and the p75 TNFα receptors (Peschon et al., *J Immunol*

160:943-952, 1998) from the CIA-insensitive C57BL/6 genetic background to the CIA-sensitive DBA/1 genetic background.

DBA/1 mice doubly deficient in p55 and p75 receptors (DBA/1 $p55^{-/-}p75^{-/-}$) were generated by crossing C57BL/6 $p55^{-/-}p75^{-/-}$ mice (Peschon et al., 1998) with DBA/1 mice obtained from Jackson Laboratories. The resulting double heterozygotes were crossed again to DBA/1 mice. The resulting progeny that were heterozygous for both mutations were further tested for homozygosity at the DBA/1 MHC complex (required for CIA sensitivity) by FACS analysis using antibodies specific for C57BL/6 and DBA/1 MHC alleles. Antibodies against the C57BL/6 and DBA/1 MHC alleles were purchased from BD PharMingen.

Those progeny that were homozygous for the DBA/1 MHC were crossed to DBA/1 for another three generations to generate DBA/1 mice heterozygous for both p55 and p75 TNF receptor mutations (DBA/1 N5 $p55^{+/-}p75^{+/-}$; "N5" refers to the fifth backcross generation). DBA/1 N5 $p55^{+/-}p75^{+/-}$ mice were intercrossed to establish a colony of DBA/1 mice doubly deficient in p55 and p75 TNF receptors (DBA/1 N5 $p55^{-/-}$ $p75^{-/-}$). In order to identify mice that were homozygous for each of the null mutations, DNA from the progeny of the latter crosses was analyzed using PCR assays specific for the murine p55 and p75 TNFR genes, using DNA extracted from ear punches.

For tracking mutations in the p55 gene, the following PCR primers were used:

p60-B: 5'-GGATTGTCAC GGTGCCGTTG AAG-3' (SEQ ID NO:9)
p60-E: 5'-CCGGTGGATG TGGAATGTGT G-3' (SEQ ID NO:10)
p60-spe: 5'-TGCTGATGGG GATACATCCA TC-3' (SEQ ID NO:11)
pgk5'-66: 5'-CCGGTGGATGTGGAATGTGTG-3' (SEQ ID NO:12)

Twenty-five pmole each of the four primers listed above were added to the ear punch DNA and the mixture subjected to 32 cycles of PCR for 1 minute at 94°, 1 min. 65° and 30 seconds at 72°. PCR products were resolved and visualized on 3 % USB fine resolution agarose (cat. # 73422) gels run in TAE buffer and stained in ethidium bromide. Expected PCR products using the above primers were 120 bp for $p55^{+/+}$, and 155 bp for $p55^{-/-}$. Heterozygous mice were expected to yield both products. Additional nonspecific products migrating at about 300-500 bp were seen occasionally in $p55^{-/-}$ mice.

The following primers were used to track the p75 mutations:

p80-Kas: 5'-AGAGCTCCAGGCACAAGGGC-3' (SEQ ID NO:13)
p80i-1: 5'-AACGGGCCAGACCTCGGGT-3' (SEQ ID NO:14)

pgk5'-66: 5'-CCGGTGGATGTGGAATGTGTG-3' (SEQ ID NO:12)

These PCR reactions used 50 pmole p80-Kas, 100 pmole p80i-1 and 20 pmole pgk5'-66 for 32-34 cycles for 1 minute at 94°, 1 minute at 65° and 30 seconds at 72°. PCR products were resolved and visualized on 3 % USB fine resolution agarose gels run in TAE buffer and stained in ethidium bromide. $p75^{+/+}$ mice were expected to yield a 275 bp product, and $p75^{-/-}$ mice to yield a 160 bp product. Heterozygotes were expected to yield both products. Additional nonspecific products were occasionally seen migrating at about 50-100 bp.

Homozygous DBA/1 $p55^{-/-}p75^{-/-}$ mice were thus identified using PCR and thereafter were interbred.

EXAMPLE 4

Collagen-induced arthritis in DBA/1 $p55^{-/-}p75^{-/-}$ mice

The following experiments using the $p55^{-/-}p75^{-/-}$ DBA/1 mice of Example 3 demonstrate that TNFα-independent CIA can be effectively treated either by administering a test agent that inhibits signal transduction by CD30 or IL-1.

CIA was induced in $p55^{-/-}p75^{-/-}$ DBA/1 mice by administering heterologous type II collagen according to the protocol described above in Example 2. Two experiments were conducted, each using 15 mice per test group. These experiments were designed to determine whether the inhibitors being tested could prevent the development of CIA in these mice. Mice were randomly divided into groups (n = 15) at the time of the boost and were injected daily with either the test agent or the control protein for 14 days.

Compared with wild-type DBA/1 mice, the DBA/1 $p55^{-/-}p75^{-/-}$ mice injected with collagen displayed a delayed onset and slower course of disease. However, significant clinical symptoms appeared in the mutant mice 15-60 days after the second collagen injection. As expected, no diminution of arthritis symptoms was observed in DBA/1 $p55^{-/-}p75^{-/-}$ mice when they were treated with p75 TNFR.Fc (ENBREL®; Immunex Corporation). In contrast, ENBREL® is highly effective in reducing arthritis symptoms in wild-type DBA/1 mice with CIA (see Example 2 above). Thus, the arthritis observed in the $p55^{-/-}p75^{-/-}$ DBA/1 mice is not mediated by TNFα.

Experiments were conducted to determine whether molecules other than TNFα played a role in CIA in DBA/1 $p55^{-/-}p75^{-/-}$ mice. In these experiments, agents that inhibit the CD30/CD30L or the IL-1/IL-1R1 interaction were tested to see if they would affect CIA in this animal model. Monoclonal antibodies against either IL-1R1 (M147;

Immunex Corporation) or murine CD30L (M15; preparation described in Example 1) were administered by intraperitoneal injection to these mice, while control mice received rat IgG. Each experimental group consisted of 15 mice. Antibody treatment was initiated at day 21 (at the time of the collagen boost), and was administered for 21 days for experiment #1, and for 28 days in experiment #2. For M15 antibody, a dose of 50 μg was administered per day, and for the M147 antibody, a dose of 50 μg was administered every two days. Clinical score was determined three times per week, using the clinical scoring system described in Example 2.

As illustrated in Table 5 (experiment #1) and Table 6 (experiment #2), the administration of M147 or M15 significantly reduced arthritis in the DBA/1 $p55^{-/-}p75^{-/-}$ mice, with M147 resulting in an almost complete amelioration of disease.

TABLE 5

Treatment	% Disease Incidence	Severe Disease	Mean Clinical Score on Last Day
M15	26%	0.06%	0.75
M147	0.06%	0%	0.07
rat IgG	67%	60%	4.27

TABLE 6

Treatment	% Disease Incidence	Severe Disease	Mean Clinical Score on Last Day
M15	60%	40%	2.7
M147	0%	0%	0
rat IgG	80%	67%	4.1

The results of these two experiments show that collagen-induced arthritis can be established in a TNFα-independent manner, and that inhibiting either the IL-1/IL-1R1 or CD30/CD30L interactions effectively reduce disease in these mice.

EXAMPLE 5

Mouse experimental allergic encephalomyelitis model

This example demonstrates the efficacy of antagonists of the CD30/CD30L interaction for treating multiple sclerosis. A mouse model for multiple sclerosis was

employed for this purpose. Chronic experimental autoimmune encephalomyelitis (EAE), which is a well accepted experimental model for this disease, was induced in female C57BL/6 mice (Taconic Farms Inc., Germantown, NY) using a modification of the protocol described by Mendel et al. (*Eur. J. Immunol.* 25:1951-59, 1995). In brief, disease induction involved the immunization of mice with the MOG35-55 peptide derived from rat myelin oligodendrocyte glycoprotein (Mendel et al., 1995). Modifications to the disease induction protocol of Mendel et al. included the use of a lower dose of MOG35-55 for immunization (see below), no booster immunization, and the use of RIBI® adjuvant instead of complete Freund's adjuvant.

To induce EAE, groups of age and weight-matched mice (11-13 mice per group) were given a dose of 100 μg of rat MOG35-55. The MOG35-55 was emulsified in 0.2 ml RIBI adjuvant (Corixa Corporation), and injected subcutaneously at three sites distributed over the shaved flank. To induce EAE with accelerated onset, the mice in a second experiment (Experiment 2) received by intravenous injection 500 ng pertussis toxin (List Biological Laboratory Inc, Campbell, CA), which was administered 48 hours after they received their dose of MOG35-55. The mice in Experiment 1 received no pertussis toxin, thus disease onset in Experiment 2 was accelerated as compared with Experiment 1.

Administration of antibody or placebo was initiated on the day after the MOG35-55 was administered (day 1) and was continued through day 11. Each mouse was injected intraperitoneally every other day with 0.2 ml pyrogen-free phosphate-buffered saline (PBS) or 0.2 ml PBS containing one of the following: (i) 100 μg M15 (anti-CD30L); (ii) 100 μg rat IgG (Sigma); or (iii) 75 μg M147 (anti-IL1R1). Endotoxin levels were <10 EU/mg of protein for all reagents. Mice were monitored daily for 35 days (Experiment 1) or 30 days (Experiment 2) for weight loss, disease onset and severity of clinical signs of EAE by an independent observer blinded to the treatment groups.

The severity of EAE was assessed using a standard EAE index system in which "0" is used to indicate an asymptomatic mouse and clinical scores ranging from 0.5 to 4 was used to indicate varying degrees of ascending paralysis. The severity of EAE was assessed using a slightly modified version of a commonly used EAE scoring system. In this system, "0" were used to indicate a mouse with no evidence of disease and scores of 1-5 were used to indicate varying degrees of ascending paralysis as follows: 1, tail paralysis; 2, hind limb weakness; 3, partial hind limb paralysis; 4, complete hind limb paralysis; 5, moribund or dead. The disease protocol described above induces an acute

episode of disease in control mice (peak score of 2-4) from which most recover at least partially. Thus the acute episode of disease is not lethal and mice do not reach a score of 5. The aforedescribed scale was modified to include a score of "0.5" which was given to mice that showed the earliest signs of EAE but that did not exhibit complete paralysis of the tail. Mice given a score of 0.5 exhibited some or all of the following symptoms: overnight weight loss of 1-2 grams; noticeable tremor when held up by the tail; and weakness at the distal tip of the tail.

The median day of onset of EAE was determined by Kaplan-Meier Survival analysis. Significant differences in onset between groups were assessed using a Log-Rank comparison. Fischer's exact test was used to analyze the statistical significance of differences in the incidence of EAE among the groups of mice.

Results of these two experiments demonstrated the ameliorating effects of either of the tested antibodies on the onset, incidence and severity of the clinical course of EAE. As shown below in Table 7, administration of either anti-CD30L (M15) or anti-IL-1R1 (M147) resulted in delayed disease onset and a reduced incidence of disease in both of the experiments.

TABLE 7

	Incidence (%)			Median Day of Onset	
Group	Expt 1	Expt 2	Combined Results	Expt 1	Expt 2
PBS	10/11 (91%)	11/13 (85%)	21/24 (88%)	22	16
rat IgG	9/11 (82%)	12/13 (92%)	21/24 (88%)	21	16
anti-CD30L	6/11 (55%)	8/13 (62%)	14/24 (58%)	35	23
anti-IL-1R	5/11 (45%)	9/13 (69%)	14/24 (58%)	not determined	25

In Table 7, for the combined results for mice that received M15 or M147, when compared with the rat IgG group, $p<0.05$ for the incidence of disease. In Experiment 1, for median day of onset for the M15 mice, $p=0.067$ vs Rat IgG and $p<0.05$ vs PBS. In Experiment 2 in Table 5, for median day of onset, $p<0.005$ vs rat IgG for both the M15 and M147 groups.

For these same two experiments, Table 8 shows the mean percent change in body weights within each group over the acute course of disease. As shown in Table 8, mice

that received either anti-CD30L or anti-IL-1R1 antibodies lost less weight during this time than mice that received rat IgG or PBS.

TABLE 8

	Treatment	Mean Percent Body Weight Change ± SEM (All mice)	Mean Percent Body Weight Change ± SEM (Affected mice only)
Expt 1	anti-CD30L	-2.0 ± 2.4*	-6.2 ± 2.7
	anti-IL-1RI	-1.5 ± 1.9**	-5.4 ± 3.2
	PBS	-7.3 ± 3.1	-9.2 ± 2.7
	Rat IgG	-9.7 ± 1.8	-11.4 ± 1.75
Expt 2	anti-CD30L	0.7 ± 2.1***	-5.3 ± 2.0**
	anti-IL-1RI	-7.7 ± 3.5*	-13.3 ± 3.7
	PBS	-16.3 ± 2.9	-19.9 ± 1.8
	Rat IgG	-19.9 ± 2.9	-21.7 ± 2.6

To calculate the weight data in Table 8, the baseline weight for each mouse was defined as its weight on day 12 (Experiment 1) or day 10 (Experiment 2) relative to immunization. These days were chosen as reference points because all of the mice in each experiment were weight-matched on day 0 and the mean weight of all the groups increased in a similar manner between days 0-12 (Experiment 1) or days 0-10 (Experiment 2). The mean weights of the various groups diverged after these time points due to weight loss associated with the onset of EAE. The left-hand column of Table 8 shows the mean percent body weight change calculated for all of the mice in each treatment group, that is, both the clinically affected (clinical score of at least 0.5) and non-affected mice were included for this calculation. The right-hand column of Table 8 shows the mean body weight change during the acute phase of the disease for *only* the mice that were clinically affected. Body weight change for the affected mice was calculated based on the difference between the baseline weight of each individual mouse and the *minimum* weight observed for that mouse after the onset of disease. For those mice that never showed clinical evidence of disease, the percent body weight change for Table 8 was calculated by comparing the baseline weight of each non-affected mouse to its weight on day 25 of the experiment.

The data in Table 8 illustrate that either anti-CD30L or anti-IL-R1 is effective in slowing the weight loss otherwise observed in mice injected with MOG35-55. The Student's t test was used to determine the statistical significance of these body weight differences. Numbers found to be statistically significant are marked with asterisks in Table 8, and p values were as follows: *$p<0.05$ vs rat IgG controls; **$p<0.01$ vs rat IgG controls; ***$p<0.001$ vs rat IgG controls.

Table 9 presents the clinical score results for Experiments 1 and 2. Group means and SEM were calculated based on the peak clinical score for each mouse (0 for non-affected mice; 0.5 to 4 for affected mice). The mean peak clinical score for each antibody-treated group was compared with the rat IgG-treated group using the Student's t test, and the p values are shown in the last column of Table 9.

TABLE 9

	Treatment	Mean Peak Clinical Score (± SEM (scale 0-4))	p vs Rat IgG
Expt 1	anti-CD30L	1.1 ± 0.3	0.0621
	anti-IL-1RI	0.9 ± 0.3*	0.0362
	PBS	2.0 ± 0.3	0.7402
	Rat IgG	2.1 ± 0.4	NA
Expt 2	anti-CD30L	1.3 ± 0.4*	0.0122
	anti-IL-1RI	1.3 ± 0.3*	0.0056
	PBS	2.2 ± 0.3	0.3262
	Rat IgG	2.7 ± 0.3	NA

As shown in Table 9, significant differences in clinical score were observed between groups of mice treated with either M15 or M147, as compared with the control mice. Differences that were determined to be statistically significant ($p<0.05$) are marked in Table 9with an asterisk.

EXAMPLE 6

Blocking CD30L delays kidney failure in a murine model of systemic lupus erythematosus

Female (NZB x NZW)$_{F1}$ hybrid mice (referred to hereafter as "NZB/W mice") spontaneously develop a lupus-like disease characterized by the presence of serum autoantibodies to double-stranded DNA (dsDNA). These mice, which eventually experience total kidney failure, are often used as a model for experimentation directed at better understanding and treating lupus in humans. Over time, this condition in NZB/W mice progresses to kidney malfunction as manifested by the appearance of proteinuria. In a trial experiment to study the onset and progression of disease in these mice, about half had serum anti-dsDNA titers and about 10% had proteinurea by 26 weeks of age. By 38 weeks of age, 20% of the mice had died, and of the remaining mice, 52% were proteinurea positive and 98% had serum anti-dsDNA titers.

Female NZB/W mice 32 weeks of age were used in an experiment to determine whether anti-CD30L could delay the development of lupus-like disease. Within a group of 32 week old mice, 98% were serum positive for antibodies against dsDNA (detected by ELISA), but only 40% had progressed to renal disease as assessed by proteinuria (detected using CHEMSTRIP®; Roche). Mice from this group that were negative for proteinuria were used in the following experiment.

Thirty-two week old NZ/B mice that did not have proteinuria were divided into two groups, and each group was treated every other day with 150 μg of either anti-CD30L (M15) (group of 10 mice) or rat IgG as a control (group of 9 mice), administered by intraperitoneal injection. Treatments were continued for five weeks, and the mice were assessed weekly for the presence of proteinuria. Approximate values for the percentages calculated from the results of this experiment are summarized in Table 10 below.

TABLE 10

Treatment	Percent Incidence Progression to Proteinuria				
	Week 1	Week 2	Week 3	Week 4	Week 5
Anti-CD30L	10%	10%	10%	10%	20%
Rat IgG	0%	10%	20%	30%	40%

The data in Table 10 shows a trend toward decreased incidence of proteinuria in mice treated with anti-CD30L as compared with control mice. A scale from 1-5 was used to assess the degree of proteinurea among these mice. At five weeks, the mean proteinurea index for control mice in this experiment was 1.7, whereas M15-treated mice had a mean proteinurea index of 0.6. This result suggests that humans suffering from systemic lupus erythematosus might benefit from treatment with antibodies against CD30L or with other antagonists of the CD30/CD30L interaction.

To confirm the result presented above, a second group of female NZB/W mice are randomly assigned to treatment groups as antibodies against dsDNA first appear in their serum. This experiment is designed to test the effects of treatment on the progression of anti-dsDNA titers and the progression of proteinurea. One group of mice is treated with 150 μg of anti-CD30L (M15), and the other group is treated with rat IgG as a control. Treatments are administered three times per week by intraperitoneal injection for a period of three weeks. Mice are monitored weekly for titers of serum antibodies to dsDNA and for the appearance of proteinuria.

In other experiments, the efficacy of anti-CD30L treatment will be tested in a model of chemically induced lupus. In this model, administration of the isoprenoid alkane pristane (2,6,10,14 tetramethylpentadecane) induces autoantibody production and immune complex mediated glomerulonephritis. One advantage of this model is that it is not restricted to a particular mouse strain. Initial experiments are underway in normal BALB/c and C57BL/6 strains of mice.